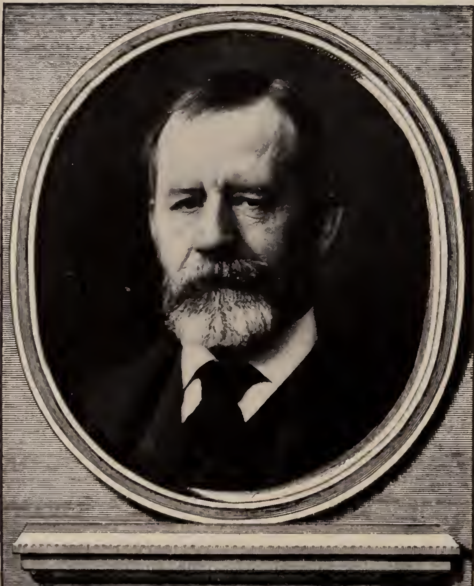

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ERRATUM.

P. 118, line 16, beneath Table, *should read* "20 to 45 minutes."

The

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A NEW STAIN FOR BACTERIAL CAPSULES WITH SPECIAL REFERENCE TO PNEUMOCOCCI.*

E. C. ROSENOW.

(From the Memorial Institute for Infectious Diseases, Chicago.)

The methods used for demonstrating the capsule of pneumococci and other bacteria differ greatly, yet all convey the idea that the capsule is extremely perishable, difficult to fix, and very soluble in water.

In a study of autolysis of pneumococci in NaCl and other solutions the simple staining methods of Welch,[1] Hiss,[2] Boni,[3] Buerger,[4] and others were found unsatisfactory in determining whether the capsule or the coccus is first to disintegrate. The differential methods of Wadsworth[5] and Buerger,[6] while more useful, were also found unreliable in this respect.

After much experimentation the following method has been devised. It has proven of such great value in a study of autolysis of pneumococci, in their identification in culture and exudates as well as in staining the capsule of *Streptococcus mucosus* and, with a slight modification, the capsule of *B. mucosus* also, that a brief report seems desirable at this time.

* Received for publication January 15, 1911.

[1] *Johns Hopkins Hosp. Bull.*, 1892, 13, p. 128.

[2] *Jour. Exp. Med.*, 1904, 6, p. 335.

[3] *Münch. med. Wchnschr.*, 1900, 47, p. 1262.

[4] *Centralbl. f. Bakt.*, 1, Orig., 1905, 39, pp. 216, 335.

[5] *Jour. Infect. Dis.*, 1906, 3, p. 610.

[6] *Jour. Infect. Dis.*, 1907, 4, p. 426

Reprint

Directions.—Make a thin smear on a perfectly clean slide or cover-glass. If the exudate, such as sputum, is too thick, add enough distilled water so that the material can be spread evenly by means of a piece of fine tissue or cigarette paper. In case of cultures (blood agar, serum—glucose agar or Loeffler's blood serum being preferable) remove a small amount of the growth from the surface of the medium and at once mix thoroughly with a loop-full of serum on the slide and spread by means of tissue paper, or, better still, make a rather dense suspension in a few drops of distilled water, and then mix an equal quantity of this suspension with serum. As the smear becomes nearly dry, cover for 10 to 20 seconds with a 5 to 10 per cent aqueous solution of tannic acid; wash in water and blot; stain with carbol- (saturated alcoholic solution gentian violet [Grübler] 1 pt., 5 per cent phenol in water, 4 pts.) or anilin-gentian-violet ½ to 1 minute, heat over flame but do not boil; wash in water again; Gram's iodine solution for ½ to 1 minute; decolorize in alcohol (95 per cent); stain for from 2 to 10 seconds, depending upon the thickness of smears, with saturated alcoholic (60 per cent) solution of Grübler's eosin; wash in water and blot; finally, clear in xylol and mount in balsam, or examine directly. (If the organism like *B. mucosus* is Gram-negative it may be stained with Loeffler's or aqueous methylene blue.)

The pneumococci are stained deeply brownish-black, sharply differentiated from the capsule, which is stained pink. Beautiful results are also obtained with the *Streptococcus mucosus.* In the thickest part of the smear the space occupied by the capsule may be perfectly clear; elsewhere in the smear, if properly made, where the conditions are suitable for absorption of eosin, the capsule is stained deeply pink; not rarely a clear retraction zone (often mistaken for the capsule in former methods) may be seen peripherally to a distinctly stained, often large, capsule.

In case of sputum in which the cocci are imbedded in a more or less tenacious mucus the capsules, at times, are not rendered stainable by the above method. In that case it is well to fix and stain simultaneously with the 2 per cent aqueous tannic acid, 4 parts, and saturated solution of gentian violet, 1 part. This modification often gives beautiful results. The cocci, however, decolorize

easily and the tannic-acid-gentian-violet may be followed by carbol-gentian-violet and then the usual procedure. Ordinary carbol-fuchsin diluted 5 to 10 times, aqueous eosin (50 per cent sat. sol.) may also be used to stain the capsule although the saturated alcoholic (60 per cent) eosin has given the best results. Decolorization after the modified Gram procedure of tannic-acid-fixed smears is more rapid than in the case of heat-fixed smears, which should be borne in mind.

The age and the completeness of saturation of the alcoholic solution of gentian violet is an important factor. Old "ripened" solutions give the best results. An alcoholic solution of gentian violet from an old stock bottle in which a considerable insoluble residue was present gave uniformly good results without the use of tannic or other acids. Smears fixed in heat, saturated aqueous solution of $HgCl_2$, Mueller's or Zenker's fluid, and less constantly formalin-fixed smears gave beautiful capsules with this stain. Most of the drawings and microphotographs were made from these specimens. Freshly prepared solutions were found unsatisfactory unless the smears were treated with an acid. The changes which take place in the ripening process in these solutions would seem to be the development of acidity, because fresh solutions act like old solutions on adding tannic or other acids and on first treating the smear by various acids, all of which, if used in the proper concentration, increase the affinity of the capsule for acid dyes provided the smear is subsequently treated with a strong basic dye such as carbol-gentian-violet or methyl violet. Just as the acids increase the affinity of the capsule for eosin, so they diminish (but all not to the same degree) the power of the coccus to retain the basic stain. The acids, in the order of efficiency, which have been tested in this way are tannic (2–10 per cent aqueous solutions), phosphomolybdic, phosphotungstic, picric (in various solutions), glacial acetic, and hydrochloric ($N/20$).

While the action of these acids is similar, their efficiency in rendering the capsule stainable, but, at the same time, not rendering the coccus Gram-negative, varies greatly. Tannic acid has been found superior to the others, especially if the smear is not previously fixed by heat or other methods. Phosphomolybdic acid

ranks second and gives excellent preparations in exudate as Smith[1] has recently shown, but is unsatisfactory for cultures of pneumococci because the coccus is rendered too easily Gram-negative.

L. Pelet-Jolivet[2] and others have shown that most staining reactions are colloidal phenomena. That the staining of the capsules of pneumococci is of this nature is indicated by the fact that the smears from cultures need to be made in an albuminous (colloidal) fluid such as serum or egg-white and that treatment in the colloidal solution of carbol- or anilin-gentian-violet or methyl violet is essential to render the capsule stainable.

The following table illustrates the importance of the colloidal solution of carbol-gentian-violet and acids in rendering the capsule stainable:

Thin Smears of Peritoneal Exudate Containing Many Pneumococci Were Fixed in Various Ways and Treated by the Following Solutions:	Coccus	Capsule	Albuminous Background
Sat. aq. sol. $HgCl_2$+carb.-gent.-violet	Deep purple	Invisible	Deep purple
Sat. aq. sol. $HgCl_2$+eosin	Unstained	Unstained	Light pink
Sat. aq. sol. $HgCl_2$+Gram iodin+eosin	Faintly pink	Unstained	Pink
Sat. aq. sol. $HgCl_2$+carb.-gent.-violet (from old al. sol. gent. violet)+Gram iodin+eosin	Deep purple	Pink	Light pink or purple
Sat. aq. sol. $HgCl_2$+carb.-gent.-violet (from new al. sol. gent. violet)+Gram iodin+eosin	Deep purple	Unstained	Light pink or purple
Heat+tannic or other acids+fresh carbol-gentian-violet +Gram iodin+eosin	Deep purple	Pink	Light pink
Tannic or other acids+eosin	Pink	Unstained	Light pink
Tannic-acid-gentian-violet+Gram iodin+eosin	Purple	Pink	Pink

As shown in the summary, acids increase the affinity of the capsule for eosin, under the conditions of the experiment, at the same time as they diminish the avidity with which the coccus retains the basic stain when treated by Gram's method. Another interesting observation is that if a smear is fixed in tannic-acid-gentian-violet, washed in water, treated with Gram's iodin, over-decolorized in alcohol and stained with eosin, capsule and coccus are stained deeply pink. If this smear is then treated a second time with gentian violet solution the affinity of the capsule for the basic dye is found to be greater than that of the coccus, which is exactly opposite to what it was in the beginning. A striking analogy to this behavior of pneumococci and their capsules has been observed

[1] *Boston Med. and Surg. Jour.*, 1910, 163, p. 791.

[2] *Die Theorie des Farbeprozesses*, 1910.

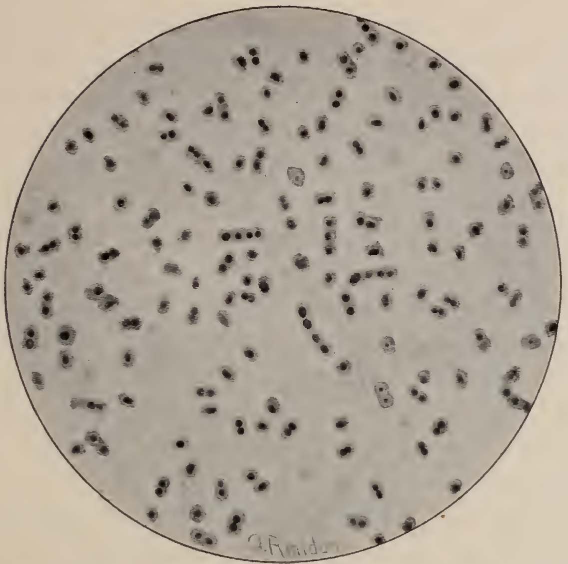

Fig. 1.—Microphotograph of field showing encapsulated pneumococci in exudate from the peri toneal cavity of guinea-pig dead of pneumococcus peritonitis.

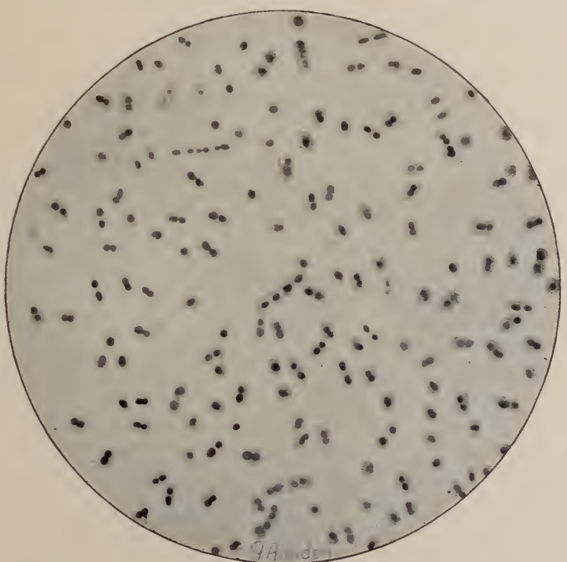

Fig. 2.—Same as Fig. 1, after fixed film was soaked in water for 24 hours; note the swollen capsules.

when they undergo autolysis in NaCl or other solutions. Up to a certain point, usually at the end of from one to three days, depending upon the degree of virulence, the affinity of the coccus for basic dyes largely disappears, it becomes Gram-negative while the affinity of the capsule for eosin increases. At this point the capsules frequently appear empty. Gradually, as disintegration continues, the affinity for eosin also disappears. These observations with the fact that capsules are easily demonstrated after repeated washings in water indicate that the capsule of pneumococcus is an integral part of the organism and not merely a precipitation of albuminous material surrounding it. But that it plays a determining rôle in virulence is unlikely, because encapsulated pneumococci may be susceptible to phagocytosis and at the same time possess no noteworthy degree of virulence.

In this connection it is of interest to point out that when pneumococci have lost their affinity for basic dyes, the result of autolysis, and have become Gram-negative and eosin staining, they seem to have lost a large part of their toxic property. When injected subcutaneously they produce a prompt rise in opsonin with no negative phase. They now absorb opsonin from serum and are relatively more susceptible to phagocytosis.

A comparison of the new method with Buerger's and other methods in a study of various strains of *Streptococcus pyogenes*, *Streptococcus mucosus*, and pneumococcus in both exudate and cultures brings out the interesting fact that what has been looked on as a capsule around the coccus in former methods is only a retraction zone of the albuminous film, the result of the fixing process. In exudates *Streptococcus pyogenes* usually contains no capsule although occasionally, as in otitis media, the cocci are surrounded by a capsule which is indistinguishable from that of the pneumococcus. In cultures, however, the method is of great value in differentiating these organisms. The streptococcus rarely forms capsules, the pneumococcus regularly, soon after isolation. But the differentiation of the *Streptococcus mucosus* from a highly virulent pneumococcus on morphological and tinctorial grounds alone, even by the aid of the new method, is not always possible. It is true that the capsule of the former, as Buerger points out, is

Fig. 3.—Third generation of pneumococci on blood agar, isolated from blood in lobar pneumonia soaked in water for 3 hours, and spread in serum.

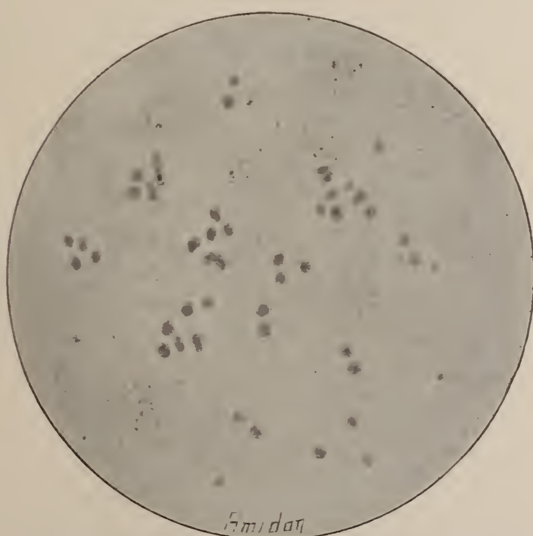

Fig. 4.—Recent isolated strain of pneumococcus grown in broth and suspended in NaCl solution at 37° C. for 24 hours. Note the many empty capsules.

usually more ragged and larger and the indentations not so regular about each diplococcus, yet pneumococci of high virulence, as Wadsworth has found, at times present similar pictures. The following facts seem to show that what in the past was taken for a solution of the capsule, when water is added after the smear was fixed on the slide, is probably simply an alteration in surface tension so that the capsule fails to take the stain under the conditions. Capsules of pneumococcus fixed by various ways and stained by the new method take the stain deeply after being "soaked" in water for several days to a week at 4° C., even after the center of the coccus has apparently disappeared. Beautifully stained capsules are given by this method when fixed (heat, chemicals) smears are washed in water for 2–24 hours and then covered with serum in a thin film and treated with tannic acid. If no serum or tannic acid is applied the capsule appears to have been dissolved, the area occupied by it being perfectly clear. During the process of autolysis in NaCl solution at 37° C., the capsule is the last to disintegrate. Welch noted lysis of pneumococci within their capsules in the exudate of resolving pneumonic lungs. I have found similar, often striking, pictures of empty capsules and cocci in all stages of disintegration inside of well-preserved capsules in pericardial, peritoneal, and pleuritic exudates, in smears of pneumonic lungs and sputum, in otitis media and mastoiditis.

Conclusions.—Contrary to the generally accepted view the capsule of pneumococci and allied organisms is not difficult to preserve nor is it readily soluble in water; and to stain it is a problem of rendering it stainable rather than one of preservation. The reactions which render the capsule stainable appear to be colloidal reactions. The capsule of pneumococci, while more stable than has been believed, is not a necessary factor in order to make pneumococci virulent.

PLATE I.

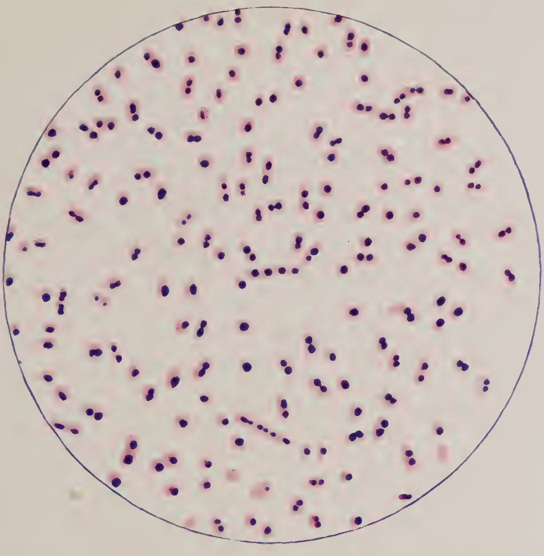

FIG. I.—Same as Fig. 2 in text. The fixed film was soaked in water for 24 hours. See the swollen capsules

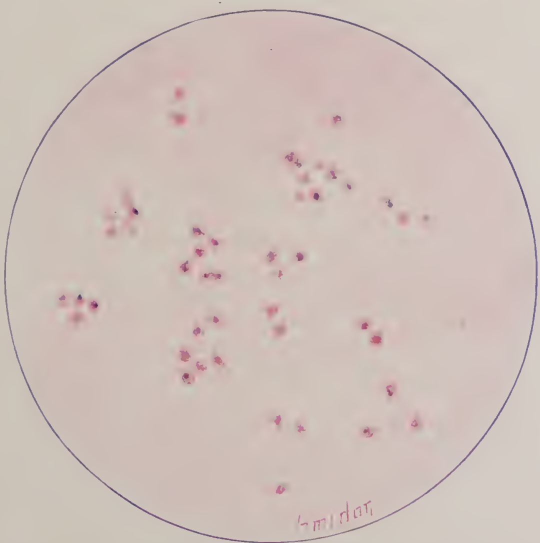

FIG. 2.—Same as Fig. 4 in text. Pneumococcus suspended in NaCl solution at 37 c. for 24 hours. Note the many empty capsules.

The smears used for the illustrations were fixed in saturated water solution of $HgCl_2$ and stained with carbol-gentian-violet, which was made from an old stock bottle of saturated alcoholic-gentian-violet, and counterstained with saturated solution eosin in alcohol (60 per cent). The picture is precisely the same when the film is fixed in tannic acid.

THE PROBLEM OF TRANSMISSION IN TYPHUS FEVER.*

RUSSELL M. WILDER.

(From the University of Chicago [Department of Pathology] and he Memorial Institute for Infectious Diseases, Chicago.)

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* Received for publication April 24, 1911.

IV.

I.

DEFINITION.

Typhus fever is an infectious disease which runs an acute course of from 12 to 15 days and culminates spontaneously in a more or less abrupt lysis. It is characterized by an incubation period of from 5 to 20 days, a high continued pyrexia, a petechial rash, is commonly considered to be extremely contagious and, although I hope to be able to show in later pages that this contagiousness is of a peculiar nature, not dependent upon the direct transmission of the contagium from the sick to the well, but rather by the transfer of body parasites (lice) carrying the contagium, nevertheless, if given the proper conditions of crowding and *vermin* the infection becomes readily communicable and spreads rapidly from individual to individual, flaring sporadically into widespread and dangerous epidemics.

II.

HISTORY AND GEOGRAPHIC DISTRIBUTION.

It would be out of place in the present paper to review in any but the most cursory manner the epidemic history of typhus fever, and the reader is referred for fuller study to the comprehensive works of Murchison and Hirsch.

The Greek term typhus (τύφος), meaning smoke, mist, or fog, was employed by Hippocrates to define a confused state of the intellect with a tendency to stupor (*stupor attonitus*) such as is found in typhus fever. The expression was used, however, with broad application until applied to typhus fever by Gaultier de Sauvage in 1760 and Cullom of Edinburgh in 1769 (Murchison). Previous to Sauvage the disease was known as pestilential or putrid fever, or by some name indicative of its certain peculiarities or characteristics, such as contagious fever, jail, army, or ship fever. A list of a hundred or more synonyms is given by Murchison. Those met with in the modern literature are: typhus, typhus fever, exanthematic typhus, contagious typhus, spotted fever, and petechial typhus of English and American authors, typhus exanthématique of the French, Fleckfieber and ansteckende typhus of

the Germans, and tabardillo or tifo of the Spanish and Mexican literature.

The disease was formerly confused with other continued fevers of similar clinical symptoms, particularly with typhoid fever, the doentheritis of Louis, and typhus abdominalis of the German authors, and it is to the credit of American medicine that a Philadelphia physician, W. W. Gerhard, was the first to insist positively and conclusively on the non-identity of these two diseases. Typhus has absolutely nothing in common with typhoid, either in etiology, in the manner of its transmission, or in its chief clinical symptoms, and Gerhard's conclusions have been repeatedly reiterated by others (Stille, Jenner, Curschmann) and generally accepted. It must be borne in mind, however, in studying the history of typhus fever that epidemics reported in the older literature as typhus may have been typhoid.

Similarly, recurrent or relapsing fever, caused by the spirillum of Obermeyer, has repeatedly been confused with typhus. Relapsing fever like typhus is prone to assume an epidemic form in times of distress and famine, and the names "famine fever" and "Irish ague" have been applied to both diseases, particularly in Ireland, where they existed side by side. The distinction between the two affections was first drawn in Ireland in 1826 and later upheld by the writings of Henderson in Edinburgh and Wm. Jenner in London. The discovery of the spirillum of relapsing fever in 1868 settled any doubt that might still have lingered.

Plague has also been confused with typhus in the history of infectious diseases. This is particularly true in ancient writings of epidemics in which the two diseases were long confounded and named alike "pestis" and "febris pestilens." Murchison believes that the plague of Athens, which broke out during the siege of that city when the population was suffering from famine and overcrowding, was typhus, and it is possible that many of the epidemics of antiquity referred to in the writings of Livy and Tacitus, and even in the books of the Old Testament as "pestilence" were typhus fever.

From this confusion of terms it is obvious that the historical study of any of the continued fevers becomes very difficult. The

difficulty is still further enhanced by the frequency with which one epidemic has accompanied another. Thus it is supposed (Creighton) that the "great plague" which depopulated London in the year 1665 was ushered in by an epidemic of typhus fever and it seems clear that nearly all of the great "famine fevers" of Ireland were mixed epidemics of typhus and relapsing fever. During the epidemic of 1846 in Ireland when nearly a sixth of the entire population contracted the contagion, three diseases, enteric fever or typhoid, relapsing fever, and typhus, occurred simultaneously.

During the siege of Granada in 1489 the Castilian army under Ferdinand was decimated by a fever, which from its character was named "el tabardiglo," "the cloak," by the Spanish authors. The fever is described by Vilaba as being accompanied by a profuse spotted eruption. The name tabardiglo or tabardillo was undoubtedly later applied to typhus fever, is indeed used today in the Spanish and Mexican literature, and it is highly probable that the disease in question was typhus fever.

The first unmistakable account of epidemic typhus is found in the writings of Fracastorius and concerns a fever which raged in Italy in 1505 and again in 1528. The disease is described both as to symptoms and contagiousness and is differentiated from the plague "febris vera pestilens" and measles. Epidemics, many of which were undoubtedly typhus, followed each other at short intervals in Europe during the following years. Ambrose Paré recorded a pestilential fever which occurred in France in 1568 accompanying true plague, from which it was differentiated. An epidemic which occurred in Verona in 1580 is described in detail by Petrus y Castro, who considered it identical with "La Pourpre" of the French, tabardiglo of Spain and the Fleckfieber of the Germans. This disease was highly contagious, prevailed in the winter months, and followed a famine. The symptoms noted are a small, weak pulse, a dry, black tongue, the face and eyes injected, delirium and stupor passing into coma, and an eruption which resembled flea bites, but could be distinguished from these, and which appeared in the fourth to seventh day of fever.

From 1619 to 1648 when Europe was in the throes of the Thirty Years' War whole towns and armies were decimated by a maculated fever, and as late as the twentieth century typhus has followed in the van of European armies. During the Napoleonic wars the disease was spread by the imperial troops over France, Italy, Germany, and Russia, while among the troops engaged in the Crimean War it wrought terrific havoc, and during the Russo-Turkish war of 1878 Michaili estimates that over 100,000 Russian troops were attacked with typhus fever. The mortality reached 50 per cent.

Since the Napoleonic era epidemics of typhus have been frequent in Ireland and England but fewer on the continent. The fever of 1817–19 in Ireland during which time 737,000 cases are recorded was probably chiefly relapsing fever (Welch, 1819),

as was also the epidemic of 1826–29. The mortality in both of these periods was very low and the exanthema of typhus seems to have been very rare (Graves). In 1836 an epidemic of undoubted typhus began in Ireland, involved the entire United Kingdom, and spread to the United States and Canada. The mortality during this epidemic amounted to over 20 per cent. Again in 1847 an epidemic of frightful extent commenced in Ireland, spread to Liverpool and London, and raged throughout England and Ireland until the summer of 1848. Like many other typhus epidemics this was preceded by extensive failure of the crops. The disease was carried in crowded ships of immigrants to America and Canada, where a series of epidemics of greater or less extent occurred during the following years (Wynne).

Epidemics in England in 1862 were attributed to disorganized industrial conditions, numerous strikes, and consequent distress among the poor. Admissions to the London Fever Hospital reached 14,000 during the following seven years. Since this date no great exacerbations have occurred in Great Britain, although small outbreaks have repeatedly been reported, particularly in Dublin, Glasgow, and Liverpool. An epidemic was reported by Dudfield, McWeeney, and others to have occurred in London in 1898; Hay reports 131 cases in Aberdeen in 1906, and Wilson in a publication of recent date mentions sporadic cases and small epidemics which arise from time to time in Belfast.

As regards Western Europe: after the Napoleonic wars typhus practically died out, and France has suffered comparatively little from the disease, even the Franco-Prussian War leaving her untouched (Chauffard). A curious epidemic referable to certain provinces of Brittany from whence it was spread to the capital and other cities by wandering vagrants is reported by Thoinot and Dubief and others in 1893. The disease was controlled in 1893 but recurred in 1894 and caused in all 149 cases in the department of the Seine, of whom 69 died.

Germany because of her proximity to Poland has been less fortunate. In 1847–48 there arose a severe epidemic in East Prussia, imported from Poland, and since this date, according to Curschmann, typhus has become endemic in Ober Schlesien and has even taken root in East and West Prussia so that Guttstadt (quoted by Curschmann) was able to collect statistics concerning 10,600 typhus patients received in the Prussian hospitals during the five years between 1877 and 1882. Of late, owing to the adoption of hygienic precautions, the number of cases in these centers has been reduced to a minimum (Leonhardt).

Russia has suffered heavily for years from typhus fever, and in 1908, following a period of political unrest, an epidemic flared up which left scarcely a place untouched (Rabinowitsch).

Our own country has been fortunately almost entirely spared from any very severe ravages of the disease. The early epidemics described by Wynne in Baltimore, Gerhard in Philadelphia, Clark in Boston, and Russell in Connecticut were relatively insignificant in size and rapidly extinguished in every instance. They seem to have been imported by foreign immigrants. The Civil War left us practically untouched by typhus. In 1863 and 1864 there occurred an outbreak in New York (Corse), and an epidemic of 39 cases occurred in Philadelphia in 1864, but Da Costa, who reports the latter event, is of the belief that it was not brought from the camps to the city. The disease, he says, was scarcely a disease of the American army and certainly not of any portion of it from which soldiers would have been apt to be sent to Philadelphia and he believes that it was imported from other parts of the world. Since

this date there are but few records of typhus in the United States, and these, with but few exceptions, concern isolated cases (Shannon, and see *Report of the State Board of Health, Michigan*, Vol. 21). An epidemic of no small significance broke out in New York City in 1880 and 1881 (Janes). The epidemic began in September, 1880, and was at first confused with typhoid fever. The contagiousness of the disease, its typical eruption and fever promptly led to a true diagnosis of typhus. The epidemic was confined for the most part to crowded lodging and tenement houses and although by the enforcement of strict hygienic measures it was soon controlled, the number of cases as recorded at the Riverside Hospital, where all were quarantined, numbered 506. The mortality equalled 24.5 per cent.

Mexico has for years been ravaged by typhus or tabardillo. According to Indian hieroglyphics translated by Spanish authors and studied by Liebermann (quoted by Chauffard) the disease reigned on the tableland of Anáhuac at the time of the Aztec Empire under the name of "masahuathl" (Chauffard), and in the last century terrific epidemics have occurred in Mexico City, notably those of 1812–14, 1824, 1839, 1846–48, 1861, 1867, 1875–77, 1892–93, and 1896. In 1893 there were 3,000 deaths from typhus in the federal district and over four times this number of cases; this out of a population not exceeding 350,000. In 1896 there were over 3,000 cases during the early spring months, and every year a toll of several hundred lives is exacted by the disease.

The typhus of Mexico is curiously confined to the central plateau and does not affect at all the lower hot country which borders the coast, a subject which will be considered later.

Epidemics of typhus are today fewer and smaller than they have been in the past, and the disease is restricted to certain regions. Here it smolders endemically, flaring up from time to time. The chief of these regions have already been mentioned, namely Dublin and Belfast in Ireland, the central plateau of Mexico, the provinces of Morbehan and Finister of Brittany in France, and Russia, particularly Poland and the East Sea provinces. Other places where the disease occurs more or less regularly are Tunis, Algeria, and Egypt in northern Africa, Spain, Hungary, and the Balkan States in the valley of the Danube, and Turkey. In India typhus has been described by Lyell and Facquhar (quoted by Husband) and more recently by Husband and Hepper. The disease here as in Mexico seems to be confined to the cooler hill country and rages most extensively in the winter months of February, March, and April. According to Husband it is endemic in the trans-Indus districts from Baluchistan to Yusufzai and Hagara, and in the Himalayan Hill tracts. In 1897 Matignon reported typhus to be endemic in Pekin and the entire north of China, the affection taking on an epidemic character of greater or less severity every year in the spring. In 1898 Yersin and Vassal published a short account of a limited outbreak of typhus in the French possessions of Indo-China. The disease is apparently unknown in the tropical parts of Central and South America, in southern Africa, New Zealand, and Australia.

III.

THE EPIDEMIOLOGY OF TYPHUS FEVER.

A correct theory of the cause of a disease should be able to explain not only its specific origin, but all the varied aspects of its

epidemiology regardless of how puzzling these may be, and before proceeding with the question of the exciting cause of typhus it is my purpose to consider the several factors of its epidemiology. Many of these may be looked upon as predisposing factors.

FACTORS WHICH INFLUENCE SUSCEPTIBILITY TO INFECTION.

There is no question of the greater susceptibility to infections of individuals and populations exhausted by famine, starvation, and distress. This is true of all diseases, but the history of typhus in particular is filled with instances of the disease accompanying and following times of misery and want. The Irish and English epidemics of the past were almost invariably preceded by failure of the crops, or periods of distress from famine or strikes (Murchison, Graves). On the other hand widespread exacerbations of the disease have occurred in Ireland independently of such influences and at times of general prosperity; witness the fever which broke out in Dundee in 1865, which was induced by the inhabitants of the surrounding country flocking into the town in consequence of work being uncommonly abundant and wages high (Pie-Smith).

Typhus fever is in general considered to be a disease of that part of the population most afflicted by poverty. An investigation of the condition of life of 18,268 typhus patients admitted during twenty years to the London Fever Hospital revealed that 95.76 per cent were inmates of workhouses or dependent upon philanthropic relief. In London typhus is almost unknown among the better classes except where there has been direct intercourse with patients. The same is true of Dublin, Glasgow, and the European cities.

In Mexico City a relatively large percentage of cases occur among the better classes, but here conditions exist which are somewhat different from those found in Europe. The poor population of Mexico is proportionately very much greater than that of London, and the streets, particularly the crowded business streets of the center of the city, are filled with poor Indians, clothed in rags and infested with vermin, who beg alms and sell lottery tickets, periodicals, and small wares of one kind or another. Indeed, as one walks along the street he cannot avoid brushing against these

people and picking up from their clothes any infection which they may harbor. All classes are crowded together in the street cars, and the public cabs are used as lounging places by the cocheros (coachmen) and their uncleanly friends. Finally it must be remembered that in Mexico the servant class is drawn from among the very poorest of the population. Domestics taken into well-to-do families come from the most squalid and disgusting "dobi" houses, and as they return to their homes for frequent visits, often to sleep at night, it is readily understood that they may serve as carriers of the disease from the poor to the rich. It is no uncommon thing to find lice in the hair and clothes of these servants. The same is true of the "portero," who is usually quartered on the first floor of the large apartment houses of the better classes. He and his family are poor people and dirty, and he visits his unclean friends in their part of the city, while they visit him in his quarters. Thus in many ways opportunities are afforded for the infection of the well-to-do and cleanly portion of the population by a disease which in other countries is ordinarily confined to the poor.

Other factors which predispose to infection with typhus by decreasing the general resistance of the individual are overwork and bodily fatigue, mental distress, worry, and loss of sleep (Murchison, Graves, Terrés). Dread of the disease is supposed to be a powerful predisposing cause by Murchison. The example of a medical student in Edinburgh is cited, who possessed such a dread of typhus that he could scarcely be induced to enter a ward, and who was one of the first students to fall a victim to the epidemic of 1847.

Finally, other diseases undoubtedly predispose to typhus. This is particularly true of acute infections such as relapsing fever and cholera, as witness the fact that large epidemics of typhus are frequently preceded or accompanied by other epidemics. As regards the individual cases it is commonly supposed that chronic diseases and alcoholism predispose to typhus. In this connection, however, it has been noted in Mexico by Ulrick that typhus cadavers which come to autopsy are singularly free from cirrhosis and other liver affections as well as from tuberculosis. This observation may of course be explained by the fact that typhus usually attacks

rather young individuals, whereas the large majority of cases in the ordinary run of autopsies are old chronic diseases.

The resistance of the individual to typhus is further influenced by the following factors:

Age.—On the relation of age to the occurrence of typhus fever all authorities are agreed. The disease is most common in young adults, and although not uncommon in children is relatively rarely met with in individuals below 15 years of age. In older adults the number of cases is fewer, but the severity of the disease increases. Murchison ascertained that the mean age of 3,456 cases admitted to the London Fever Hospital during the ten years 1848 to 1857 was 29.33 and Rabinowitsch in the epidemic in Kiew found that 1,895 cases out of 3,099 occurred between the ages of 15 and 30. These figures do not differ materially from those of other infectious diseases, excluding children's diseases and typhoid fever, in which the mean case age is considerably lower. It is clear that that part of the population which is above 20 years of age, granting equal susceptibility to the disease, is more exposed to the danger of picking up a communicable infection than are children and older people, who are more protected.

Sex.—According to all the European authors sex plays no rôle in influencing susceptibility to typhus. In general the number of cases among men and women is about equal. In an epidemic of 3,099 cases in Kiew in 1908 (Rabinowitsch), 2,383 were males. This preponderance of males is attributed to the greater number of men in the prisons from which most of the cases were taken and also to the greater personal cleanliness of the women. Thoinot in epidemics observed in Brittany in 1893 recorded a considerably greater number of cases among the women, which he attributed to the absence of many of the men in the fishing boats and to the exposure of the women to infection by their duty of nursing the sick.

In Mexico, on the other hand, there seems to be a constantly greater proportion of cases among the men. A glance at the accompanying Chart II on which are graphically recorded the number of male and female cases in the Federal District for the period 1893 to 1906 reveals a constant majority of men, a majority

which is relatively greater than the majority of male inhabitants. Sr. Dr. Terrés in his scholarly exposition of the etiology of tabardillo calls attention to this fact and comments upon it to the effect that this greater proportion of male cases is not what should be expected in an easily communicable disease such as typhus, in which the women who are employed in nursing the sick are more in contact with patients than the men. But Terrés disregards the fact that a very large number of the cases in Mexico City arise in the armies and the prisons, where relatively few women are exposed to infection, and that the total number of male patients is bound to be swollen by this account. In general it would seem that men and women are equally susceptible to the disease.

Idiosyncrasy.—It is a strangely curious fact that many individuals show an unusual immunity to typhus fever. Cases are on record of physicians and nurses who have been daily exposed to infection for years and yet have never been taken ill. On the other hand individuals may succumb on the first exposure. Certain individuals have taken the disease after several years of immunity to constant exposure. Murchison mentions the case of one of the engineers at the London Fever Hospital, whose duties took him daily into the typhus wards and included cleansing of the dirty bedding, who died of typhus contracted for the first time after fifteen years of service.

It is a rather commonly accepted idea that highly intellectual people are more susceptible to typhus fever, and it is certain that the disease assumes in them a more severe form, the nervous symptoms, convulsions, delirium, and coma being greatly exaggerated. The mortality among this class of patients runs very high.

Racial immunity.—Whether residence in an infected district or constant exposure to infection of a mild character increases the resistance of an individual to typhus as it seems to do in the case of typhoid fever is doubtful. Physicians and nurses acquire no immunity from daily contact with the disease, as is evidenced by the fact already noted that many eventually succumb after months or years of exposure. On the other hand it is certain that the European and American population of Mexico City is more sus-

ceptible to typhus than are the native inhabitants, and this seems to indicate that a racial immunity to the disease may be acquired.

The greater part of the foreign population of Mexico City lives in the eighth ward, the Quartel VIII. This quartel is a clean and prosperous quarter of the city, and is composed in large part of well-to-do individuals of naturally cleanly habits, well nourished, and dwelling in modern and sanitary houses. It is true that in the same quartel there may be found a large number of poorer people, but in general the more prosperous condition of this section of the city is apparent. On the other hand Ward II is made up of the poorest and dirtiest people of the city, where all conditions seem to conspire to produce a situation most favorable for the spread of typhus, and yet we are confronted with the astonishing fact that there occurs approximately as much, even, according to Terrés, relatively more typhus in Ward VIII than in Ward II.

A greater susceptibility of the inhabitants of Ward VIII over those of Ward II would alone explain this apparent anomaly, for, although the well-to-do classes residing in Ward VIII are far from protected from exposure to infection, as mentioned, nevertheless in Ward II the population is so much denser and the people live in such crowded and filthy conditions that the opportunities for the transfer of the disease from one to another must be much greater. Ward VIII is a new and rapidly growing section of the city, and a large part of its population has never been exposed to typhus, while Ward II is peopled almost exclusively by Mexicans of the lower class, who for years and generations have lived in the midst of typhus fever. A considerable proportion of these have had the disease and recovered and are consequently immune, and the possibility that many others have passed through mild and unrecognized infection cannot be excluded. But further than this, typhus fever during the countless epidemics that have ravaged Mexico in the past has killed off a very large number of people. In each of these epidemics the less resistant of the population succumbed, and thus gradually a racial resistance to the disease may have become established.

The statistics of the American Hospital of Mexico City indicate a much greater mortality among the American and foreign patients

of that institution than is observed among the natives of the Mexican hospitals, and this even in the face of the greatly superior nursing and care received by the patients of the American institution. Nearly all of these are private cases, and the rules of the hospital necessitate their employing special trained nurses. On the other hand, the statistics collected at the Hospital General and the Hospital Juarez concern charity ward cases who are given little individual attention and the poorest of nursing. And yet the mortality among the Mexicans rarely exceeds 20 per cent, while that observed in the American hospital has been 34 per cent.[1]

Immunity conferred by previous attack of typhus.—Recovery from a single bona-fide attack of typhus fever apparently grants immunity from subsequent infection. This has been generally believed for years and is confirmed by the results of experiments performed on monkeys, to be described later. Instances of two successive attacks of the disease are rarely authentic, although some appear in the literature which apparently cannot be discredited. Murchison never observed a single instance of a nurse or patient at the London Hospital having had an unequivocal second attack with eruption, which in his opinion is a much rarer occurrence than a second attack of variola or scarlatina. Jacquot (quoted by Murchison) employed hospital attendants in the Crimean War who had had typhus and in no instances were any attacked again.

Most of the physicians of Mexico City seem to be of the opinion that a second attack of the disease is quite possible. Dr. Escalona informs me that he himself has had two attacks, but in general it must be admitted that the immunity of an individual who has recovered from typhus fever is quite as complete as that following typhoid, measles, or smallpox. In any of these diseases the immunity conferred by one attack occasionally becomes exhausted, at least in particular individuals. A good example of this is seen in diphtheria. Although most people have but one attack of diphtheria, an occasional individual is encountered who suffers from repeated attacks.

Occupation.—There is probably no truth in the assertion that

[1] These statistics were given me by Miss Wilson, the superintendent of the American Hospital of Mexico City. The number of cases of typhus fever treated from 1888 to January, 1910, was 144, of whom 50 died.

butchers and tanners enjoy a peculiar immunity to typhus fever, and in general occupations play no rôle in the epidemiology of the disease, excepting in so far as they expose to infection. Thus those who attend the sick and are naturally in constant danger of infection, as physicians, nurses, and priests, are frequently attacked with typhus. In older days when hospitals were not maintained up to the standards of the modern institutions as regards cleanliness, when baths were unknown, and patients were crowded into ill-kept barracks, physicians and nurses were particularly prone to contract the disease, and the fatalities in these professions from typhus fever became appalling. In Ireland during the epidemic of 1847, no fewer than 500 physicians took the fever, of whom 147 died, and in an epidemic which was carried to Breslau by the retreating troops of Napoleon, 18 out of 40 of the total number of doctors of the town died. In the Crimean War 58 deaths from typhus occurred among the 400 military officers of the French army (Thoinot).

Of peculiar interest in the light of the possibility of insect transmission of typhus fever is the relative frequency with which those who handle the clothing of patients contract the disease. Repeated mention is made in the literature of the susceptibility of laundresses to typhus. In Mexican literature Terrés quotes O. Galvan to the effect that the only case of infection in the hospital of Lagos, which harbored 243 typhus patients, occurred in the laundry. Several cases broke out in a steam laundry of Mexico City while we were studying the disease in that metropolis.

FACTORS WHICH FAVOR THE DISSEMINATION OF THE DISEASE.

Overcrowding.—Typhus fever is a disease of cities, armies, ships, and prisons, and the overcrowding of human beings is of undoubted importance in the propagation of epidemics by facilitating the transmission of the disease.

Among the earliest accounts of jail or "gaol" fever which from description permit of recognition as typhus are those of the "black assizes," which occurred in England during the sixteenth and seventeenth centuries and have become historical. In the assize at Exeter, in 1586, 38 Portuguese sailors were tried. These men

had been captured some time before and cast into "a deep pit and stinking dungeon" in Exeter Castle. A contagious disease broke out among them and at the trial was communicated to several of the attendants of the court. The judge and many others died of the fever, and an epidemic started which spread widely. The disease, from the description of the symptoms, was undoubtedly typhus. Those exposed at the court did not develop fever until fourteen days later, indicating an incubation period which corresponds with that observed in typhus.

Another equally grewsome assize occurred in "Old Bailey" in 1750. A hundred prisoners, many of whom were ill at the time of the proceedings, were on trial. These were seated at the bar or confined in two small rooms which adjoined the courtroom. The court was crowded to excess, so that the air became exceedingly vile. Within a week or ten days following the trial a large number of those who had been present, including the judge and other dignitaries of the court, were seized with a fever characterized by a weak pulse, delirium, and petechiae, which lasted for two weeks. Over 40 persons died.[1]

A dozen noticeable instances of jail fever are recounted by Murchison, and more recent epidemics still emphasize the proclivity of typhus for prisons. A large number of the patients received in the hospitals of Kiew during the epidemic of 1908 came from the prisons which were "shamefully crowded" (Rabinowitsch). In Alexandria the disease is frequently epidemic in the jails, where it always shows great contagiousness (Sandwith).

The common city prison of Mexico City, the "Carcel de Belem" (Bethlehem), seems to be a perfect brood-oven for typhus. Its characteristics are overcrowding, poverty, and filth. Its inmates are chiefly composed of the lowest and most miserable of the population, people with absolute disregard for personal cleanliness, abhorrent of baths, clothed in soiled and vermin-infested rags. These people come from crowded and insanitary homes. On their arrival the dirtiest are bathed, but the number so treated is but a fraction of the whole, and even those receiving a bath are dressed again in the same filthy clothes to mingle with the other prisoners.

[1] See Charles Creighton, *A History of Epidemics in Britain*, 1891.

Later they are required to wash their clothes more or less regularly, but a condition of cleanliness is never even approximated.

The population of Belem varies between 4,000 and 4,500. The edifice is very old, and was founded as a refuge for indigent religious women in 1683. Additions have been made since that time, but in general the buildings are those of a century or two ago, the walls massive, the ceilings low, and the rooms poorly ventilated. No statistics are available concerning the floor space allotted to an individual, but it cannot exceed 4 square meters. The courts, corridors, and wards are literally packed with human beings, and during our visits I was shown cells not exceeding 12 by 8 feet occupied by five or six prisoners. The more dangerous criminals are confined in these small cells.

The infirmary accommodations in the prison are admittedly insufficient. The women's ward is an ill-ventilated, dark, low-vaulted room of dimensions not exceeding 50 by 30 feet. The walls and floor are of stone. On the occasion of my visit the dozen beds in the room were all filled, and patients were lying on the floor. This was not at the time of an epidemic. Contagious cases, including typhus, are taken to the Hospital Juarez as soon as recognized, but as no quarantine is held over them while in the prison infirmary, and as typhus cases are not diagnosed earlier than the fourth or fifth day of their sickness, abundant opportunity is open for the communication of their disease to others.[1]

Under such circumstances there is small wonder that typhus occurs constantly in Belem. During many epidemics 80 and 100 patients have been removed from its walls daily, and while we were pursuing our investigations in the city in the winter and spring of 1910, a non-epidemic year, there occurred in the prison, in January, 32 cases, in February, 130, in March, 43, and in May, 16 cases. These statistics are obtained from the records of the Hospital Juarez. Records of previous years reveal that at no time does the disease ever die out in the prison, and that occasionally epidemics flare

[1] In justice to the director of the prison and others concerned in its administration it should here be mentioned that the shortcomings of the institution are thoroughly appreciated by them and by the city and government authorities; that efforts are being made to improve the conditions in Belem, and that work has been already begun on the building of a new general prison which is to accommodate 6,000 inmates. This institution is to cost 12,000,000 pesos and will be as thoroughly modern as is the present Mexican penitentiary, a model institution.

up in Belem quite independently of those in the city. Thus in October of 1908, at a time when there was relatively little typhus in the city, there were removed from Belem to the Hospital Juarez, on the third, 17 cases, on the fifth, 12, on the seventh, 74, and on the eighth, 28 cases.

The importance of Belem as a center for the perpetuation and distribution of typhus will be considered again later. Its inmates return to all parts of the city on leaving the prison, and if there is such a thing as carrying typhus in one's clothes, either in the form of the virus or of carrying insects, Belem must be regarded as an important distributing center.

Further evidence of the influence of crowding and congestion upon typhus fever is the well-known prevalence of the disease in ships and armies, which has earned it the names "ship-fever" and "military fever." The overcrowding which is so likely to occur in the ships and barracks is probably the chief factor in ship and military fever, but it must also be remembered that other conditions favorable to the growth of epidemics are commonly present in armies and navies. Among these may be mentioned neglect of personal cleanliness, bodily fatigue, exhaustion, and poor food.

Furthermore, it is widely recognized in Europe that typhus commonly affects to the greatest extent the most congested sections of the city. In Edinburgh it has practically remained restricted to the wretched and crowded parts of the "Old Town," even at times of the greatest epidemics. The same can be said of London and Dublin, and Gerhard, speaking of the epidemics which broke out in Philadelphia in 1836, says that the disease first occurred in the filthiest and most crowded parts of the city. A similar restriction of the disease to the poorer and more congested parts of Philadelphia was true of the epidemic of 1866 described by DaCosta. Cases were met with among those living in comfortable circumstances, but the greatest ravages took place in the southeastern section among the poor.

The same segregation of the disease to the poorer localities of the city does not apply quite so strictly to Mexico City, where typhus continuously occurs in every quartel, and where, as stated, relatively as many cases arise in the prosperous Ward VIII as in the most

crowded and miserable Ward II. The explanation of this apparent anomaly is to be found, I believe, first, in the greater susceptibility to typhus fever of the foreigners who comprise a large part of the population of Ward VIII and, second, in the abundant opportunity for exposure of the better classes to contact with the poor, both of which conditions have been discussed.

Neglect of cleanliness.—Monjaras believes that pauperism constitutes one of the principal etiologic sources of typhus, and personal squalor and filthy apparel—attributes of poverty and congestion of human beings—are universally conceded to be predisposing factors of typhus fever. More will be said about this later, but an interesting example of their importance may properly be cited at this point. In the history of the epidemic which occurred in France in 1893 and 1894, the importance of "vagabondage" in keeping up and spreading the disease is repeatedly emphasized by those writing on the subject. Typhus had been endemic in Brittany for some time, but in 1891 and 1892 it assumed an epidemic exacerbation in the commune of Carnoit. Its spread from here was later traced step by step along routes followed by wandering tramps and vagabonds to Paris, which was reached by the disease in 1893. In all of the cities along the routes the resorts of vagrants—prisons, police prefectures, "refuge communal," and "asile de nuit"—were first attacked, and in Paris typhus was first noticed in the prefecture of police. For the most part the epidemic in Paris was confined to vagrants and habitués of the commonest lodging places, whose regard for personal cleanliness was at a minimum. Aside from these the only persons who took the disease were some of those whose duties brought them in contact with the sick—physicians, nurses, and attendants (Thoinot and Dubiet, Proust, Deschamps, Spillman).

In this connection the report of Richter on an epidemic of 58 cases in Marienburg, West Prussia, in November, 1893, is of interest. The author comments on the fact that the first two cases appeared simultaneously and were both vagabonds. Shortly thereafter other cases arose in West Prussia which at first could nearly always be traced to a popular tavern in Marienburg.

Cases of typhus have arisen persistently in Berlin for years, but

are confined to certain of the poorest inns, the resorts of tramps and indigents.

In the case of the Paris epidemic the prefecture of police which had been such a pesthole of contagion ceased to be dangerous and did not produce a single case after the day that a thorough disinfection was made and a rule enforced requiring all new arrivals, no matter what their class or state of health, to subject themselves to a bath and to the disinfection of their clothes.

CLIMATOLOGICAL FACTORS.

Typhus is in general a disease of the temperate and cold climates, as indicated by its geographic distribution (see p. 15). In India, as has been mentioned, the disease is confined to the hill country (Husband), and in Mexico, where temperate climate prevails on the central plateau and hot near the coast, it is restricted to the former. The altitude of the plateau of Mexico varies from 4,000 to 11,000 feet, and in Mexico City, elevation 7,500 feet, the temperature never becomes oppressively hot even at midday, and the nights, summer and winter, are chilly or cold. In contrast to this temperate climate the conditions in the cities bordering the coast and in the deep valleys at the edge of the central tableland are quite tropical. The heat during the greater part of the year is intense, and cold weather, although occasionally felt in Veracruz during a "norther," is rare and of short duration.

Dr. Iglesias, Dr. Macias, and other physicians of Veracruz, members of the Board of Health of that city, were consulted concerning the absence of typhus fever. All of these gentlemen assured me that the disease is never known to develop in the city. A careful watch has been kept for the last few years over the whole population in order to guard against yellow fever, and an endemic case of typhus has never come to the attention of the board, although an occasional case is imported into Veracruz from the plateau. Dr. Iglesias has seen several soldiers who have come down with typhus shortly after the arrival of their regiment from Mexico City. These men were cared for at the military hospital without any attempt at isolation, and in no instance did contagion occur or the disease spread to others.

In Jalapa (altitude 4,000 feet), which lies just below the edge of the plateau, Dr. Canovas y Pasquel, attending physician of the general hospital of that city, informed me that he had not seen or heard of a single case of typhus except such as were imported from the higher country. Most of these come from the near-by town of Perote.

Terrés comments at length upon this freedom from typhus of the *terre caliente*, and according to this author a line may be drawn paralleling the coast and circumscribing the elevated tableland. The altitude of this line approximates 1,800 meters (6,000 feet), and all the typhus of Mexico is restricted to the region above it.

This geographical limitation of endemic typhus in Mexico is probably of climatological significance, although other factors may enter into the problem. The traveler voyaging from the capital is impressed shortly after his arrival at Orizaba and the hot country by the greater personal neatness and cleanliness of the poorer population over that of Mexico City, and he learns on inquiry that the people bathe frequently and pay great attention to the cleanliness of their apparel. Many are dressed in white cotton garments. The body lice, which are universally distributed among the poor Indians of Mexico City, and tolerated without repugnance, are here looked upon with disgust and horror, and although occasional vermin-infested individuals may be found, they are quite rare.[1]

But besides paying more heed to personal cleanliness the natives of the *terre caliente* live as a rule in better sanitary conditions than those of the plateau. The dwellings of the poor are built of straw and poles, are well ventilated, and as a rule fairly neatly kept. Also food in abundance is found, and famines such as prevail on the tableland are unknown. These factors deserve consideration, but none of them can be considered as of primary importance in limiting typhus to the plateau.

In temperate climates where winter is distinguished from summer, typhus is a disease of the winter and spring, a decrease

[1] Dr. Iglesias, in Veracruz, was unable to obtain any *Pediculi vestimenti*, the white body louse, for experimental purposes, and Dr. Garcia experienced the same difficulty. Dr. Canovas informed us that he seldom if ever found body lice on the entrants of the general hospital of Jalapa. The head and pubic lice are more abundant, although even these are relatively far less prevalent than among the natives of the plateau. The importance of this scarcity of lice I desire to consider later. Bedbugs and fleas are numerous in the hot country.

in the amount of the disease invariably occurring in the summer months. This is true of England, of France and Russia, of Tunis (Conseil), of Egypt, and of India (Husband), and as a rule has been attributed to the greater crowding of people indoors during the winter months, and the consequent greater opportunity for the communication of the disease from one person to another.

The same predilection of epidemics of typhus for the winter and spring months of the year is observed in Mexico (see Charts I and II), and annual exacerbations which almost always assume epidemic proportions commence usually in November and persist until the late spring. Here, as in Europe, the greater crowding encouraged by the colder weather undoubtedly facilitates the spread of the disease. However, the seasonal changes are relatively slight, and the cold ceases long before the decline of the epidemic. Another factor must be considered in Mexico, where the typhus season besides including the colder winter months is the *dry season*. A strange relationship seems to exist between the duration of the typhus epidemic and the beginning of the rains, as well as between the amount of typhus in any given year and the relative degree of drought. The rainfall which is abundant during the remainder of the year decreases to a minimum during the months December to May, the period of the greatest prevalence of typhus. The fields are arid, the ditches which are running with water during the rainy season are empty, and in March, April, and May clouds of dust are blown into the air by the high winds. The onset of the rains corresponds roughly to the decline in the epidemic, as may be seen by a glance at the accompanying charts.[1] Furthermore the amount of typhus which will develop in any given year seems to be indirectly proportional to the amount of rainfall of the preceding rainy season. Pruneda calls attention to the fact that in 1905, preceding the severe epidemic of 1906, the rainfall was decidedly less than the average (compare Chart II), as 423.07 mm. is to 581.9 mm.; that furthermore in this year the rains began late, and heavy precipitation was delayed until September. Terrés is firmly convinced that an abundance of rain in any given year indicates less typhus for the following year, and that the reverse

[1] It is worthy of notice that the number of cases of typhoid in Mexico City increases with the advent of the rainy season.

occurs after long continued drought, but does not hazard an opinion as to the reason for this relationship. Monjaras in San Luis Potosi was able to see but little connection between the amount of rainfall and the annual exacerbation of the disease.

IV.

THE PROBLEM OF THE TRANSMISSION OF TYPHUS.

We are now in a position to consider the actual means of transmission of typhus fever from one person to another.

CLASSIFICATION OF INFECTIOUS DISEASES.

Infectious diseases are commonly classified into contagious and non-contagious infections. In the former class belong smallpox and diphtheria, which are ordinarily considered as "catching" and are communicable either by immediate contact of individual with individual or indirectly through the intervention of "fomites," such as bedding, drinking cups, and clothing. *Contact* is the essential factor in the transmission of contagious infections.

In the case of the non-contagious infections, examples of which are typhoid and cholera, this contact is not essential. Typhoid may be transmitted by milk contaminated with the typhoid bacillus to individuals all living widely separated from one another and far distant from the patient who originally supplied the infecting germs. In cholera a contaminated water supply may spread the disease throughout that part of a city which is supplied by the infected water, leaving the other sections of the town unharmed, as happened in the case of the epidemic in Hamburg in 1892. In anthrax and tetanus the micro-organism may live for years outside the human body and finally, invading a susceptible host, produce sickness. These diseases may, it is true, be communicable by contact, and nurses attending typhoid patients not uncommonly contract typhoid, but they are relatively far less likely to be thus communicated than are the so-called contagious diseases. Therefore a disease is usually classed as non-contagious if it is possible to eliminate contagion by precautions designed to prevent the invasion of the body by the specific germ. Thus in typhoid,

if a nurse exercises scrupulous care in handling the excreta of her patient and excludes all possibilities of contaminating her own food, either by soiled hands or by the use of dishes from which the patient has eaten, she need have no fear of herself contracting typhoid; the disease is not "catching."

There exists a third class of infectious diseases, namely those which are transmitted by the sting of insects. Attention was first called to this method of infection in 1893 by the work of Theobald Smith and Kelbourne on Texas fever, an infection of cattle, which was shown to be caused by a pear-shaped protozoon (*Pyroplasma bovis*) and to be transmitted from animal to animal by a certain tick (*Boophilus bovis*). Subsequently insects have been found to transmit malaria (*Anopheles*), yellow fever (*Stegomyia fasciata*), and the sleeping sickness of Africa (the tsetse-fly, *Glossina palpalis*). The spirochaetae of relapsing fever have been demonstrated in bedbugs which are collected from the beds of patients, and monkeys have been infected with relapsing fever by exposing them to the bites of infected bugs. The spotted fever of the Rocky Mountains is transmitted by the wood tick (*Dermacentor*), and the reports of recent plague investigations in India emphasize the prominent part played by the flea in carrying plague from rat to rat and from rat to man.

Such insect-borne diseases may or may not be communicable by direct contact and without the intermediation of insects. Plague is an example of this kind. The pneumonic form of plague is readily spread from one person to another by contagion. However, most insect diseases are not "contagious" and are commonly transmitted by the bite of one specific insect, and in no other manner; witness the success attending the prophylactic treatment of yellow fever directed against the mosquito in Havana, the Panama Canal Zone, and Veracruz.

Nothing in the epidemiology of typhus supports the theory that it is transmitted by water, like typhoid or cholera. Some authors, particularly Mexican, have advanced the supposition that decaying vegetable and animal matter or the accumulation of the excreta of human beings propagates the disease (Mendez), but there seems to be no reliable evidence in favor of such a view.

THE CONTAGIOUSNESS OF TYPHUS.

Typhus has always been considered to be one of the most contagious of all diseases. Hare, in his *Practice of Medicine*, expresses the opinion that exposure for a considerable time to the atmosphere of a room which is poorly ventilated and which contains typhus patients is the most effective way of contracting it. Curschmann regards the disease as highly contagious in the beginning and probably during all stages of the fever. Pie-Smith considers that the most conspicuous factor in the etiology of typhus is its contagiousness. Murchison is very emphatic on the matter of contagiousness, and Graves, Tweedie, Gerhard, and others all believe in the extreme transmissibility of typhus by contact.

The Mexican writers on tabardillo are less positive as to its contagiousness. Thus Bernaldez writes: "On many occasions in the course of my duties as sanitary inspector in the City of Mexico I have had to see typhus patients, and I have found that for six or eight days or sometimes for even a longer time several relatives had lived in the same room as the patient without any of them being attacked, and out of 1,089 cases reported by the medical inspectors of the wards of the city only 110 appear arising from contagion, and these all in persons of the lowest class who are extremely careless of the rules of hygiene." Chico reports that during a period of 20 years in Guanajuato only two physicians contracted typhus, and Warfield comments upon the lesser contagiousness of Mexican typhus to the European disease described by Murchison and others.

Now there are several respects in which the Mexican form of typhus fever differs from the European disease, and it may be that the former is less readily communicable than the latter, but in the light of my own experience with Mexican typhus I cannot agree with the authors quoted that it is not readily communicated among people living in crowded circumstances and infested with vermin. In the Mexican General Hospital during the six months in which we were pursuing our investigations in that institution not less than eight persons were infected. These included three nurses, one medical student, an ambulance porter, and three of the six men who were at the time pursuing investigations of the

disease in the hospital. Of course it may be objected in regard to these investigators that they were exposed to sources of infection other than the contagion of the hospital, but the incidence of the other five cases cannot be denied. Furthermore, from many of the patients who were examined by Dr. Ricketts and myself histories of exposure to previous cases could be obtained.

The belief in the contagiousness of typhus by European authors is firmly held and based on strong authority. The chief reason for this belief is the susceptibility to infection of those exposed to contact with the sick. This we have seen illustrated by the especial susceptibility to typhus of physicians and nurses. A second strong argument in its favor is the frequency with which epidemics can be traced from case to case and from point to point. "An outbreak which occurred at Carlisle, in 1871, was found by Dr. Heysham to have started from a particular house in Richard Gate. One of the persons afflicted there was a weaver, who on his recovery communicated the disease to his fellow-weavers in a large workshop and by them it was spread all over the town" (Pie-Smith). Thoinot and Calmette were able to trace nearly every case in epidemics studied by them in the Ile Tudy of Brittany to previous patients. Hlava, in a tabular record of 2,639 cases of typhus, draws the conclusion that the spread of the infection occurs only by direct contact. In a typhus outbreak of 30 cases which occurred in London in 1899 the epidemic was traced to a single family (Waldo).

Apparently the disease is highly communicable, but that this communicability differs from the contagiousness of diseases like diphtheria is indicated from the occurrence in the literature of such observations as the following:

Overcrowding is considered by the early authors on typhus as almost *essential* to its production. Thus, to quote from Murchison, "although scarlet fever and small-pox are propagated by overcrowding and defective ventilation, epidemics of them commence and spread irrespective of these influences. It is not so with typhus, which never becomes epidemic except under circumstances of overcrowding and bad ventilation."

Great importance is set upon personal uncleanliness and filthy clothes as predisposing factors to typhus by Murchison, Graves, and others. Filthy apparel is commonly vermin-infested, and it is interesting in this connection to note the repeated comments (Bancroft, 1811, Murchison, Terrés, and others) upon the exemption from typhus fever of the naked negroes in slave ships. These poor creatures were crowded

for weeks below decks in foul, stinking holds and yet, though they suffered terrifically from dysentery and other ills, they were spared from the very disease which from analogy might be expected to cause the greatest trouble, namely "ship fever" or typhus. Their escape was not due to racial insusceptibility, for negroes are quite as susceptible to typhus as whites. This was shown by the epidemic of 1836 in Philadelphia, in which a majority of cases occurred among the blacks (Gerhard).

Another illustration of the importance of clothing in typhus infection is found in the following observation of Perry of Glasgow (quoted by Graves). The fever wards of the Glasgow Royal Infirmary were two, one for acute and one for convalescent patients. Typhus, smallpox, scarlet fever, and measles were admitted. In the acute ward the patients were confined in bed and "were not allowed the use of their clothes." Now it was found that while they remained in the acute ward none of the smallpox or other patients contracted typhus fever, but that when they were sent into the convalescent ward, where they necessarily mixed with the typhus convalescents who here were allowed to dress in their own clothes, "almost all seized the typhus in an intervening period, never less than eight days." Later these smallpox and measles cases were kept in the acute ward until well and discharged, and not one caught the disease.

"Sir R. Christison, speaking of the medical students who had contracted typhus at the Edinburgh Infirmary, and who had been *attended at their own homes* by himself and two of his colleagues during thirty-two years, remarks: 'I am sure I am within the limit when I say that we have attended 280 cases of this kind, that 1,200 persons must have been more or less exposed in attending on them, and only one instance of communication is known to have occurred.'" Murchison himself comments: "How different is scarlet fever in this respect!" It is to be presumed that the homes of these students were far cleaner than the charity wards of the hospitals of that day.

More recent authors are even more emphatic as to the non-contagiousness of typhus in *clean surroundings*.

Robinson and Potts (1905) in a report on 600 cases of typhus fever in the Liverpool City Hospital comment as follows: "The patients were kept in large wards with abundant fresh air; they were bathed on admission and their clothes disinfected, with the result that no cases of hospital infection occurred." Dr. Russell of the Liverpool Fever Hospital claims that the wardmaids and nurses of that hospital only very rarely contract typhus. Out of 800 cases of typhus during the past three or four years no doctor or medical student took the disease, a condition which contrasts most favorably with the records of former times and is to be explained by the scrupulous cleanliness of the modern hospital ward (Hay).

Gotschlich in a report from the Government Hospital of Alexandria, Egypt, states that typhus frequently occurs epidemically in the prisons of that city. These prisons are filthy and vermin infested, and the disease in them always manifests great contagiousness, nurses, physicians, and attendants being attacked. In marked contrast to this condition is the absence of any cases of contagion or spread from patients treated in better hygienic surroundings, in the hospital or home, this in spite of the fact that where the patient was nursed in his own family the opportunities for contact transmission were greatly enhanced, the relatives being in most intimate daily association with the patients and even kissing and hugging them before and after death. Gotschlich has never seen a single case of contagion in families in comfortable circumstances.

Conditions in Mexico resemble those in Alexandria. As has been mentioned, Belem, the city prison, is a hotbed of infection, and in the charity hospitals, the General and the Juarez, where the cleanliness of the patients and the disinfection of entrants is only laxly enforced, cases of typhus are constantly arising among the nurses and others whose duties take them in contact with the patients.[1] Yet we have been informed by numerous physicians of Mexico City that instances of contagion are met with with extreme rarity in the families of the well-to-do, where the patient is treated at home and under cleanly conditions. Also in the private hospitals which are maintained under modern ideas of hospital sanitation instances of infection practically never occur. During the last twelve years there has not been a single house infection from typhus in the American Hospital, although in this period 144 cases of the disease have been treated. In the same period three nurses have been infected with typhoid fever while attending typhoid patients.

Finally there have come to my attention the following instances in which very intimate exposure to infection failed to transmit typhus fever.

W. N., a boy, Irish, but born in Mexico City, ran a fairly mild course of typhus fever with petechial eruption. The mother, who was not immune, slept in the same bed with the child during the nights following the third and fourth days of his fever, and for the rest of the time that he was ill, although restrained from sleeping with him, remained in very intimate contact. She did not contract the disease. The boy was a patient of Dr. Schmidtlein of Mexico City.

M. R., an American visitor at Mexico City, slept in the same room, although not in the same bed, with a typhus patient during the first three days of a typical typhus fever from which the patient later died. He remained in perfect health during the following three weeks in which he was under observation. The room inhabited by these men was clean and free from vermin.

Anderson and Goldberger cite the following: "F. J., adult, American, non-immune. Lived at a hotel in Mexico City, but came in daily intimate contact with

[1] Typhus patients who arrive at the General Hospital are sent directly to the "tifo pavilion" instead of being previously bathed and cared for at the reception ward as are all the other entrants. This custom is recognized to be highly reprehensible by the physicians of the typhus department, but insurmountable difficulties have been in the way of correcting the condition. Arriving at the typhus pavilion, the patients are bathed and their clothes taken from them, but the bathing and undressing take place within the pavilion, and any insects which may be in the clothes have ample opportunity to be brushed off on to the floor or walls. Thus the pavilion is being constantly reinfested with vermin. But still worse is the reception of the patients that arrive at night. These are put to bed in the ward, still wearing their soiled clothes, and frequently when their beds are examined in the morning the sheets and blankets are found to be swarming with lice.

cases of tabardillo between November 22 and December 16, 1909. On the nights of January 5 and 6 he slept in a bed that had been occupied on January 2, 3, and 4 by a patient in the first three days of a well marked attack of tabardillo. None of the bedding or bed clothes had been in any way disturbed in the interval prior to their use by this individual. At the end of three days the bed clothes were changed, but with this exception the bed and room remained as they had been when occupied by the patient. F. J. inhabited this room for three weeks longer. On careful search no insects other than fleas were found in the room. During a period of observation of 17 days this man continued in his usual health.

Instances of indirect transmission of typhus, that is transmission by means of intermediaries, well persons, who carry the disease to others without themselves succumbing to infection, are very numerous (Curschmann) and it is easy to understand how this would be possible either under the insect theory or under the "contagious" theory, but in this connection it is interesting to read the following: "There are no instances on record where a medical man has been the medium of transmission of typhus to his patients or to his family, as may happen in the case of scarlet fever or smallpox" (Murchison).

TRANSMISSION OF TYPHUS BY INSECTS.

The belief that insects carry typhus from man to man is of relatively recent birth, although it is held by many physicians who are in daily contact with the disease and has been voiced by several writers on the subject (Eichorst and Sambdon). Fleas and bed-bugs were long ago looked upon with suspicion by certain older Mexican physicians, foremost among whom was Dr. D. Francisco Marten.

Granting that typhus is a disease that is commonly, or at least frequently, carried about in the clothing and that it is an insect disease, three possible kinds of vermin immediately present themselves as open to suspicion. These are the flea, the bedbug, and the louse.

The flea.—This insect has been incriminated in plague as a carrier of that disease, and as typhus fever resembles plague in various clinical respects it is not unreasonable to expect that the flea may transmit the former as well as the latter. Typhus and plague are both septicemias, that is the blood in both has been

shown to be infectious on inoculation, and hence it might be expected that any blood-sucking insect could transmit either disease. Also Hay's observations in Aberdeen, which were detailed above, point toward the culpability of the flea in typhus, and yet it must be said that the flea theory does not harmonize with the etiological factors of typhus fever as we now know them.

Typhus is universally a disease of temperate climates and reaches its greatest epidemic exacerbations in the cold seasons of winter and spring, but the flea is found in greatest abundance in the tropics, and in temperate countries the flea season is not the winter, but the summer. In Mexico City during the yearly exacerbation of typhus from November to May fleas are relatively scarce; indeed, during the last year in January and February, the months in which the typhus curve reached its greatest height, great difficulty was experienced in obtaining a sufficient number of them for experimental purposes. On the other hand, in the summer when the number of cases of typhus is at a minimum fleas abound. In Tunis lice abound in the spring and early summer, the typhus season, while fleas and bedbugs are rare or absent (Nicolle). In India also it is reported that fleas almost disappear in the winter when typhus is most prevalent (Husband), and this is undoubtedly the condition in other countries.

In the second place the striking distance of typhus is short (Murchison), a condition which scarcely harmonizes with the astonishing agility of the flea. Finally, typhus is almost exclusively a disease of the poor, but in countries in which fleas are at all abundant, as for example Mexico, they are rather generally distributed among all classes of the population.

Unfortunately experimental investigation of the rôle of the flea has not progressed far. Toussaint in 1906 attempted to infect himself with fleas which he had previously fed on typhus patients. The experiment was negative; no symptoms were produced other than a temporary local infection of the skin at the point of attachment of the insects. The fleas used were collected from a cat. The experiment of Dr. Ricketts and myself with the macacus monkey and human fleas (*Pulex irritans*) will be detailed below. It consisted in the inoculation of a monkey with an emulsion of

the entire bodies of 10 fleas, each of which had been fed repeatedly on typhus patients. The monkey was not infected.

The bedbug.—Husband in India and both Gotschlich and Sandwith in Egypt incline toward the opinion that the bedbug (*Cimex lectularius*) plays the chief rôle in the distribution of typhus. Husband's arguments are as follows: Fleas are excluded because of their scarcity during the typhus season. The drabis (muledrivers) commonly harbor lice, but these insects cannot live long apart from their hosts, while the contagium of typhus undoubtedly does. Furthermore cases continually occur among army prisoners, and the enforced cleanliness of these renders them free from lice. Bugs are active all the year, attach themselves to bedding and furniture, and feed repeatedly from fresh hosts. Furthermore it has been shown that the species of bedbug common in Europe (*Cimex lectularius*) is found in the northern frontier provinces, but not in the rest of India, a distribution which curiously coincides with that of typhus fever, which is rarely met with in the "down country" (Husband).

Husband's observations seem very conclusive, but theoretical considerations also argue against the theory that the bedbug is of importance in transmitting typhus. Typhus fever is a disease of a certain season of the year, but bedbugs are active throughout the year. Now it has been rather conclusively proven that bedbugs transmit relapsing fever (Tictin, quoted by Ricketts), but relapsing fever fails to show any such seasonal variations as does typhus. Leonhardt in a historical account of typhus and relapsing fever in Breslau, Schleswig, presents two very instructive tables, the one recording the case totals of typhus fever from 1856 to 1894 and the second the monthly case totals of relapsing fever for the period 1868 to 1894. These tables, which include a very large number of cases of each disease, reveal that the case totals of typhus constantly increase during the winter months and decrease in the summer. This is true both in epidemic and non-epidemic years. In relapsing fever the cases are about equally distributed through all four seasons, and in 1868 and 1869 by far the majority of cases are confined to the months from May to August, the season when typhus is at its lowest ebb. Rabinowitsch likewise

notes the absence of seasonal variation in recurrent fever and argues from this that the summer must have some rather concrete restricting influence on typhus.

In the second place, the bedbug is only a temporary parasite of man and is rarely carried about in the clothing, but the clothing of typhus patients is usually infectious, and numerous instances have been cited in the foregoing pages to illustrate how frequently the contagion of typhus is carried on the bodies of intermediaries from one person to another.

Were the bedbug the chief factor in the spread of typhus we should expect the disease to have more the characteristics of a "house disease," but such is not the case. The large majority of the patients which were studied in Mexico could not be traced to any typhus house, and it has been noted by numerous European authors that if a patient is removed from his home fairly early in the disease subsequent house infections rarely arise (Murchison, Hay).

Again, bedbugs, like fleas, are rather generally distributed among all social classes. Typhus is confined to the poor, but bedbugs may be found in the homes of well-to-do persons, where the disease is an extreme rarity and where its spread from one individual to another is practically unknown.

A further argument against both bedbugs and fleas is to be found in the geographical distribution of typhus in Mexico. Both of these insects are quite common in the hot countries bordering the coasts, but typhus is limited to the plateau.

An experiment which was performed for the purpose of determining whether the bedbug could carry typhus will be detailed later. I was unable to transmit the disease to the monkey with these insects.

The louse.—Without further study we cannot of course exclude the possibility that either the flea or the bedbug or both may under certain circumstances act as carriers of typhus, but in the light of the etiological conditions of the disease it seems improbable that either of them can play a very important rôle. Certainly their part is far less significant than that of the louse, which I now wish to consider.

The louse has been looked upon with suspicion by various authors, among others by Netter and Thoinot, who in their "Rapport Général sur le typhus en France" discuss the possibility of transmission by this means (Nicolle). Sambdon, in an article on "Rocky Mountain Spotted Fever" in Albutt's *System of Medicine*, suggested the possibility that the louse may carry typhus, but there were apparently no direct affirmations of the importance of the louse previous to the experimental researches of Nicolle in Tunis and Ricketts and myself in Mexico City.

In 1909 Charles Nicolle succeeded in infecting a chimpanzee with typhus fever and was then able by inoculation with the blood of the chimpanzee to infect other monkeys of an inferior species (*Macacus sinicus*). Later, in conjunction with Compte and Conseil, he successfully transmitted the disease from one macacus to two others by means of human lice (*Pediculus vestimenti*) applied in the following manner:

Twenty-nine lice were placed on the skin of macacus No. 1 in the third day of his fever. Twenty-four hours later and again on each of the following days they were fed upon the two monkeys A and B. A was bitten for 6 successive days by 15, then 12, 31, 8, 6, and 3 lice, and B for 12 days by 14, then 15, 13, 9, 5, 6, 5, 4, 2, and 1. These monkeys, after rather long incubation periods of 25 and 40 days respectively, both succumbed to high fevers which in the case of the first, after an irregular course of twenty days, culminated in death. This monkey was quite perceptibly ill after the thirtieth day following his exposure to the lice, but failed to show an eruption. The autopsy findings were negative, the spleen being small. The general symptoms noted in the case of Monkey B were few; there was some weakness and loss of appetite with the elevation of temperature, but on the sixth day after beginning of his sickness a macular eruption was observed. Six monkeys were inoculated with the blood of A and B. Of these, five ran rather mild courses of fever which were difficult to interpret, but the sixth, inoculated from Monkey B, developed a typical typhus.

In experiments performed in Mexico City during December, 1909, and January, 1910, Anderson and Goldberger undertook to transmit typhus from human patients to the macacus with lice.

One of these animals showed a slight rise in temperature 8 days after its last exposure to the bites of the infected insects. This febrile elevation persisted for only two days and as it was but a trifle above the normal range for the rhesus monkey it was not interpreted as fever. Unfortunately no immunity test could be given to the animal at the time. It is very probable that the elevation of temperature observed was due to typhus.

The experiments of Ricketts and myself, the protocols of which are included in a later section, prove, I believe, to a reasonable degree of certainty, that the infected louse may by its bite transmit Mexican typhus fever or tabardillo. Thirteen monkeys of the species *Macacus rhesus* were the subjects of experiments on louse transmission. Of these, eight were exposed to the bite of infected lice, that is, lice which had been fed on human patients for two or three days before the beginning of the experiment. Five of these monkeys were infected with typhus fever by the lice. In two other experiments transmission by lice from monkey to monkey was obtained, and two "scarification experiments," which consisted in the subcutaneous introduction into monkeys of the intestinal contents of infected lice, resulted in positive transmission. Thus, in all, nine monkeys were infected by lice.

Infected lice have been shown by experiment to retain their ability to produce the disease for a period of at least seven days and the result of one experiment seems even to indicate that the infectivity of the mother louse may be inherited by her young. It is evident therefore that the louse may actually be infected by the typhus germ and that this insect may by its bite transmit the disease to a well individual. Under these circumstances it is highly probable that the usual manner of transmission of typhus fever is by the louse, especially since all of the epidemiological factors of the disease, as we now know them, point to and may be explained by such a theory.

The habits of the louse agree with the etiology of typhus and clear up the peculiar nature of the supposed "contagiousness" of the infection. Emphasis has already been laid on the short striking distance of the typhus contagion, its predilection for the clothes and bedding of the patients, and its apparent absence after

patients have been undressed and bathed. Human body lice are blood-sucking parasites and are only rarely and accidentally found away from the bodies or clothing of human beings. Their movements and habits are sluggish; they secrete themselves during most of the time in the folds and seams of the clothing, and make only rare and short excursions to the skin for food. It is only under conditions of rather intimate contact that they are passed from host to host. On the other hand, they are rather active in seeking a new host after they have been removed from their source of food, as for instance with the discarded clothing of a patient, and this activity explains the particular danger of contracting typhus of those whose duties require them to receive and undress typhus patients or to handle such discarded clothing preliminary to its disinfection.

Attention has been called in preceding pages to the restriction of typhus to the lowest and filthiest classes of the population. The body louse is limited to people of this class. As a rule lice are regarded with disgust in homes and families where fleas and bedbugs are tolerated, and even approximately cleanly habits will render people free from lice, inasmuch as bathing and the regular washing of clothing is incompatible with the life of these insects. It is true that cases of typhus occasionally occur among the better classes, but all such cases can be traced to contact with louse-infested persons. This was the observation made by Hay, who is quoted below (p. 47). In Mexico City it is not uncommon for well-dressed persons of perfectly cleanly habits to find an occasional louse on the clothing, after a walk through streets crowded with poor people, and hence, as might be expected, there occurs relatively more typhus among the better classes of that city than is described by the European authors.

Of still greater interest is the almost exact concurrence between the geographic distribution of lice in Mexico and the distribution of typhus, of which mention has already been made. *Pediculus vestimenti* is almost never found in the "terre caliente," or hot country, which is free from typhus, while on the typhus-infested plateau it is very abundant.

The absence of body lice in the hot country is due to several

factors. In the first place the habits of the people of this part of the country are more cleanly; the warmth of the climate and the abundance of water encourage bathing, and the clothing worn is made of light material, frequently white cotton, which can be washed.

Of equal or greater significance is the heat of the lower country, which appears to be unsuitable for the life of the louse. I am disinclined to insist too strongly upon this point because of the scantiness of evidence at hand, and yet such evidence as there is all indicates that the louse does not thrive at high temperatures, and I feel convinced that further observation will bear out this statement.

During the course of the experiments conducted by Ricketts and myself in Mexico City lice were used almost constantly, and at times it was found very difficult to keep the insects alive. This difficulty increased to a great extent during the warmer months of March and April, and it became necessary to keep the lice at a low temperature, 16 to 20 degrees Centigrade. Several experiments were performed in order to determine the effect of heat on their vitality. Groups of lice were placed in a thermostat, at a temperature of 35° C., death from desiccation being guarded against by supplying abundant moisture. In every instance this temperature resulted in the rapid death of the entire group, indeed a few hours at 35° usually sufficed to kill. The protocols of one such experiment are as follows:

Effect of Temperature on the Longevity of the Louse.

Lice of group 16 (*Pediculus vestimenti*) were collected from the clothes of children of a neighboring school and were presumably normal. Two hundred healthy adults were given a feed on normal monkey No. 8. They were then divided into four groups of 50 each (A, B, C, and D), each group being folded separately within a piece of muslin and placed in a cotton-plugged test-tube. The tube containing group A was put in a large air-tight jar containing a water-soaked sponge for moisture and incubated in a thermostat at 35° C.

Tube B was placed in a similar moist jar, but kept at the room temperature, which reached a maximum of 20° C.

Tube C was kept at room temperature without moisture.

Tube D was kept without moisture at a temperature of 10° to 12° C.

Sixteen hours later all the tubes were examined:

Tube A, all lice dead.

Tube B, 1 louse dead.

Tube C, 3 lice dead.

Tube D, 1 louse dead.

The difference between the room temperature of 20° and the temperature of tube D, namely 10° to 12°, had no appreciable effect, but the mortality of 100 per cent in tube A is quite striking. Some of the lice in B, C, and D were alive at the end of the fifth day, although not fed in the interval, while 16 hours at the incubator temperature proved fatal for the first group.

Nicolle, Compte, and Conseil also comment on the effect of high temperature on the viability of the louse (*Ann. de l'Inst. Pasteur*, April, 1910), and these authors found it necessary to keep the insects at a temperature below 24° C.

Anderson and Goldberger had a similar experience (*Pub. Health Report*, February 18). Lice were carried in bottles on the body, at a temperature approximating that of the body, with the result that at the end of 36 hours, although fed in the interval, all had died. The authors also comment on the significance of the influence of temperature on louse longevity in view of the limitation of typhus to the Mexican plateau.

It is a fact frequently noticed by the natives of Mexico that louse-infested individuals who go from the plateau to the warm coast countries rapidly lose their body lice independently of bathing or washing. I have been frequently told of this occurrence, although I have not had an opportunity to confirm the correctness of the information. In any case the temperature of the coast cities frequently reaches 35° C. even during the winter months, a temperature sufficient to kill the lice in the incubator; indeed it is not uncommonly still higher in these parts, and for several hours of every day the heat is more or less intense. It is therefore not unreasonable to suppose that temperature plays an important part in limiting the geographic distribution of lice and thus indirectly of typhus fever.

It is furthermore probable that temperature determines the exacerbations in Mexico by the effect of the summer heat on the number of lice.

The experiments of Ricketts and myself were continued from December, 1909, to the end of June, 1910. In the early months there appeared to be no difficulty in obtaining an ample supply of body lice for the work, but as the warmer spring months from

March on progressed the servants who were paid to collect lice became less prompt in delivering the required number. I was also told by several of the nurses at the General Hospital that the poor applicants were relatively freer from vermin during the hot period following the winter months than at any other time of the year.[1]

The coincidence between the decrease in typhus and the beginning of the hot season is remarkable, as reference to Chart I will indicate. It is of greater importance to my mind than the relation between the rains and typhus indicated on the same chart. As is shown, the typhus curve always begins to fall about the time the rains *commence*, but this is before precipitation has reached any effective amount. The typhus curve begins to drop in April, and yet until the end of May the roads remain dusty. The ditches are empty until June or July. Then, but not until then, is the influence of the rains manifested. Water becomes abundant, clothes are washed, and bathing may even be indulged in, with the result that a great destruction of lice occurs, a large proportion of the total number of infected insects is killed, and the typhus curve drops still further. When the rainfall of any given year is scanty, as for instance in 1900 and 1905 (see Chart II), the ditches are not filled, a smaller number of lice are destroyed, a greater number of infected lice are left alive, and the following year the exacerbation becomes a severe epidemic. This I believe to be the relation between the rain and typhus, its effect secondary and accessory to that of the heat, their combined effect being directed against the louse population.

It is not my intention to insist too strongly on this point, namely that the effect of heat and rain on typhus is accomplished by destruction of lice, and I recognize that other factors enter in, that the greater heat of the summer encourages better ventilation

[1] This matter is of sufficient importance to justify verification. The question could probably be settled in the course of a year or two by employing two or three reliable individuals, typhus immunes, to examine the clothing of all arrivals at some large hospital and to form by count as accurate an estimate as possible of the number of lice harbored by each individual. It would be difficult to make such an estimate absolutely accurate because of the minute size of the newly hatched lice, but even rough estimates if sufficiently numerous and extensive would, I believe, reveal, first, that more people harbor lice during the cold winter months than in the summer, and second, that the average number of lice per individual louse-carrier decreases in the hot months.

Nicolle reports that the relative number of lice in Tunis is very low during the non-typhus months of September, October, and December, whereas these insects abound in the spring and early summer when typhus is at its height.

of homes and less crowding, and that the richness of the harvests and the consequent nutrition and state of resistance of the poor is dependent on the amount of rain, but overcrowding, poor ventilation, famine, and destitution, although important accessory forces in determining the occurrence and spread of typhus fever, are not essential to its occurrence and spread, while the presence of body lice in particular is essential.

It should also be recognized that the effect of heat may be directed not so much against the life of the louse as against the duration of the infectivity of the louse, as is the case in plague in India. The infectivity of the flea is diminished or destroyed by the excessive heat of the Indian midsummer. Fleas thrive at this time, but are not infective. The possibility that similar sterilization of infected lice may be produced by the intense midday heat of the Mexican summer has not been investigated to date, but certain theoretical considerations make this seem highly probable. Chief among these is the following:

Travel between Mexico City and Veracruz is quite constant, and, as was mentioned before, typhus cases are not infrequently imported into Veracruz from the plateau. Opportunities are therefore numerous for spreading typhus from one city to the other and yet they have never resulted in the production of an epidemic in Veracruz. Indeed, I was informed by numerous physicians of that city that they had never known of a single case contracted from an imported typhus patient, although many of these were vermin-infested on their arrival, and no attempt was made toward isolation or disinfection. Lice are surely not killed quickly enough by the heat of Veracruz to safeguard the contacts of these patients, but sterilization of the lice may and seemingly must occur. Experiments designed to investigate this interesting problem have been planned and it is highly desirable that they be performed.

SUCCESS ATTENDING PROPHYLAXIS DIRECTED AGAINST INSECTS.

As a final argument supporting the theory of louse transmission is the successful result which has attended prophylactic measures directed toward the elimination of body vermin. In an epidemic which occurred in Aberdeen in 1906 insect transmission, particularly

transmission by the flea, was suspected by Hay, who gives the following reasons for arriving at this conclusion:

1. Every case of typhus examined in the hospital exhibited flea bites, these being carefully distinguished from petechiae.

2. Every case, however clean and free from body vermin himself, was found to have been in contact with vermin-infested patients at the probable time of infection.

3. There occurred no instances of contagion in families of perfectly cleanly habits, although typhus patients had lain in the house during the greater part of their illness and in two cases without attempt at isolation. One of these cases concerned a mother who slept with her child during his illness without catching the infection, a case similar to the one cited on a previous page.

4. Every nurse and wardmaid at the City Hospital who was infected was engaged in receiving and cleaning typhus patients and thus exposed to the vermin on the patients' clothing. None of the nurses who attended the patients in the wards and no physicians contracted the disease, although they were in constant proximity to the patients.

The prophylactic measures instituted by Hay were directed against the flea, but it will be seen that they are so designed as to be equally effective in destroying other body vermin such as lice.

When a patient was reported and removed from a house, the house was thoroughly fumigated with sulphur, in order to stupify all insects. The bedding, clothing, carpets, etc., were then removed to the disinfecting station, the woodwork, walls, and floors after a thorough spraying with formaldehyde were washed and the walls stripped of paper and limed. After such precautions no case of house infection occurred and the epidemic died out.

Hepper reports the successful combat of a little outbreak of typhus which sprang up in the prison hospital of Peshawar in India. In previous years the hospital had been visited by severe epidemics, and for this reason the place was rebuilt several years ago along lines designed to eliminate vermin. Iron beds and clay bunks were substituted for the wooden furniture used before. In spite of these precautions, after an interval of several years, typhus again appeared in the hospital. The iron beds were examined and

found to harbor bedbugs. These beds were then surrounded by leaves of sugar cane which being fired subjected them to a short but intense heat. The tile floor of the hospital was likewise covered with cane and fired, while the patients were removed to tents and given clean clothing and bedding. The epidemic was completely stamped out. The only other case which occurred was that of a patient who came down with the disease six days afterward and had undoubtedly been infected before the disinfection. No sick attendant of the typhus cases got the disease, which "was contrary to the usual experience, the sick attendants as a rule being very liable to become infected."

In this connection the experience of Robinson and Potts should again be referred to. Patients were bathed on admission to the Liverpool City Hospital and their clothes fumigated, with the result that no case of hospital infection was observed. The fact that this is the general experience in all modern and strictly clean hospitals is the strongest kind of an argument against the reputed "contagiousness" of typhus fever. Under cleanly conditions typhus is not contagious.

CONCLUSIONS.

1. Typhus is an insect-borne disease.

2. The disease is contagious only in so far as infected vermin are transmitted from host to host by contact and is not contagious under conditions in which vermin are excluded.

3. Three insects should be regarded with suspicion as possible conveyers of the infection. These are the flea, the bedbug, and the louse.

4. The epidemiology of typhus indicates the possibility that neither fleas nor bedbugs ordinarily play any great rôle in transmission.

5. The body louse (*Pediculus vestimenti*) stands incriminated, first by the experimental results of Nicolle, Compte, and Conseil in Tunis, and Ricketts and myself in Mexico, and again by the epidemiology of the disease.

6. The louse theory is in every way consistent with the etiology of Mexican typhus so far as this is understood and explains several

important phases of the epidemiology which were formerly obscure. Chief among these are, (*a*) the geographic limitation of typhus to the central tableland of Mexico, (*b*) the seasonal variation of the disease and the effect of temperature and rain on the epidemics in Mexico City.

7. Prophylactic sanitary measures directed against typhus fever should take into primary consideration the body louse.

V.

EXPERIMENTAL OBSERVATIONS ON TYPHUS FEVER AND ITS COMMUNICATION.

Inoculation experiments have been attempted with the blood of typhus fever patients in a wide range of animals. Mosler (quoted by Pie-Smith) injected fresh blood from patients into the veins of dogs without result, and numerous other negative experiments of the kind are reported in the European literature. Zulzer alone found that when he took blood while the disease was at its height and injected it into rabbits these died within two or three days. The symptoms were not, however, very characteristic of typhus, and the results have failed of confirmation by other investigators.

In Mexico extensive inoculation experiments undertaken by Director Gaviño of the Bacteriological Institute and his assistants failed to produce any evidence of infection in guinea-pigs, rabbits, white rats, and mice. The blood was taken from patients at different periods of the disease. Numerous other investigators have obtained similar results.

Experimental transmission from man to man has been attempted, but the results are not satisfactory. In 1876 Moczutkowski inoculated himself with blood from a typhus patient and 18 days later developed a fever and the typical symptoms of the disease. The experiment is reported in a publication of 1900. It is not very conclusive. The author claims to have had exanthematic typhus before when thirteen years old, and on several previous occasions attempted unsuccessfully to infect himself with blood. At the time of the experiment reported he was engaged in active work in the typhus ward, which exposed him presumably to the constant danger of infection by natural channels.

In 1907 M. Otero, a physician of San Luis Potosi, Mexico, made several attempts to infect human beings by inoculation. In three cases he obtained no result, but in a fourth he was successful. A volunteer was injected intravenously with 0.5 c.c. of blood taken from a patient in an early stage of a typical typhus fever of moderate severity.

Yersin and Vassal in French Indo-China have recently announced the successful

infection of two men, native coolies, by the subcutaneous inoculation of 0.5 c.c. of typhus blood. This blood was drawn from a patient in the second day of illness. In respectively 14 and 21 days after the inoculation the two coolies developed fever which began acutely, persisted for 11 days, and reproduced with great clearness all of the symptoms of the natural disease excepting the eruption. No exanthem of any kind was visible. The authors discuss the diagnosis of the disease and differentiate it from relapsing fever, dengue, and kala-azar, but no mention is made in the report of any precautions taken to prevent the accidental infection of the two subjects.

The first successful animal experiments were performed by Nicolle of Tunis, who in 1909 transmitted typhus fever from a patient to a chimpanzee by the inoculation of blood. After a latent period of 24 days the animal developed a fever of seven days' duration, which the authors interpreted as typical of typhus. Death followed the consequent cachexia. An eruption appeared on the 17th day after the injection. This consisted of rose spots and was confined to the face. A similar eruption is reported by the authors in subsequent experiments, but has been denied in their later publications (*Compt. rend.*, July, 1910).

From the chimpanzee the infection was transmitted by blood inoculation to a monkey of the species *Macacus sinicus*. Previous attempts at direct infection of the macacus with human blood had failed,[1] but in experiments undertaken subsequent to the investigations on Mexican typhus, described below, successful infection directly from man to the macacus monkey has been obtained by the Tunis investigators. In these later attempts numerous monkeys have been inoculated. Several of these were of the species *Macacus sinicus*. Rhesus, sinicus, the inuus, and the cynomologus monkeys have been used. The quantity of blood injected has varied in amount from 0.5 c.c. to 10 c.c. and the injections have been made subcutaneously in some cases and intraperitoneally in others. The sinicus has been found more susceptible than the rhesus, although individuals of both species have been successfully infected. The authors claim that typhus blood is infectious at all stages of the disease even before the onset of fever and for several days after crisis.

In December, 1909, Anderson and Goldberger, working independently of Nicolle, succeeded in transmitting Mexican typhus directly to inferior monkeys from man. Two animals, one a *Macacus rhesus* and the other a *Cebus capuchinus*, were inoculated on successive days with large quantities (10 c.c. doses) of blood from typhus patients. Both animals suffered severe attacks of typhus which were clinically typical except for the absence of eruption. The incubation periods noted were 11 and six days respectively. Immunity tests given to these animals after their recovery were resisted completely. Passage was also successfully accomplished with blood taken from the

[1]Nicolle states in his more recent publications that his failure to infect the inferior monkeys directly with human blood in his earlier attempts is to be attributed to the use of insufficient blood. In this early attempt two monkeys, one a cynomologus and the other a sinicus, were injected, each receiving 1 c.c. of blood taken from different patients during the early part of severe fever. Neither of the animals manifested any discomfort following the injection, but it was found some months later that they had been immunized by the blood. They proved resistant to inoculations of infected blood from other monkeys. This result appears to be of the nature of a vaccination or active immunization. Such an interpretation is indeed made in the publication of April, 1910, *Ann. de l'Inst. Pasteur*, p. 259: "Une inoculation de sang humaine typhique au bonnet chinois ne l'infecte pas, mais le vaccine contre lé virus de passage par bonnet chinois." It is however contradicted in a later report (*Compt. rend. de l'Acad. des Sci.*, August, 1910), in which the statement is made that a first attack of typhus fails to confer immunity if not sufficiently severe. "Une atteinte légère de la maladie expérimentale ne donne pas l'immunité."

animals at the height of their fever and the disease reproduced in other rhesus monkeys. A third passage failed.

McCampbell has also succeeded in giving the Mexican disease to monkeys by injections of blood from human patients.

Gaviño and Girard of Mexico City have succeeded in infecting a monkey of a species indigenous to Mexico, the *Ateles vellerosus*.

Prieto of Mexico City has recently reported the experimental infection of dogs with typhus fever, but to my knowledge his observations have not been confirmed by others.

PERSONAL INVESTIGATIONS.[1]

During the course of the investigations of Mexican typhus undertaken by Dr. Ricketts and myself twelve normal monkeys of the species *Macacus rhesus* were inoculated either with blood or serum from early cases of typhus. All proved susceptible to the inoculation excepting one (the animal receiving the smallest dose, No. 21), whose history follows shortly. Three other monkeys, likewise macacus, were injected with virulent monkey blood; in addition five monkeys, previously the subjects of insect experiments, proved susceptible to inoculations of human typhus blood. In all, 20 monkeys have reacted to the injection of virus in practically the same manner, and, as all were later reinoculated and proved immune to the subsequent reinoculation or "immunity test," I feel justified in concluding that they were infected with typhus by the original injection of blood. From these experiments the following conclusions may be drawn concerning the nature of typhus fever in the *Macacus rhesus:*

The monkey is not very susceptible to infection with typhus. First, the minimum infective dose has been found to lie between 0.2 c.c. and 1.0 c.c. Second, it has been found difficult if not impossible to maintain the infection by passage from monkey to monkey. Anderson and Goldberger, and McCampbell were all equally unsuccessful. All report a diminution in the severity of

[1] The following experiments were largely designed by Dr. Howard T. Ricketts, who died of typhus while engaged in this study. They were made in Mexico City during the winter and spring of 1910, were temporarily interrupted after the death of Dr. Ricketts, and were later completed in so far as was possible. Many of our results were unavoidably lost, and much work that had been planned was necessarily abandoned. The studies have not been completely reported in the preliminary communications published by Dr. Ricketts and myself, and it is the purpose of the present paper, first, to collect the substance of all of our investigations on transmission, the problem which is undoubtedly of the greatest practical interest in connection with the disease, and, second, to draw from these studies certain general conclusions. In the pursuance of these investigations we were greatly aided by the courtesy and kindness of the Mexican physicians, and by the numerous privileges granted us by the Mexican government.

the disease after the first passage, which is probably significant of an attenuation of the virus. Our own experience, although less conclusive on this point, will be discussed later.[1] It is undoubtedly safe to say that the monkey is less susceptible than man.

The incubation period after injection of the virus into a monkey occupies from six to 10 days, although it has been as short as four days and in two cases was protracted to 15 and 16 days respectively. This agrees fairly well with the incubation period observed in man. The duration of the fever extends from eight to 15 days, although in one or two mild cases it has been shorter (five days). Frequently after the first two or three days of high temperature a drop to normal is noticed which is followed by a return of fever. The course in man is almost always longer and except in sporadic cases the fever is relatively higher and more constantly sustained. No prodromal symptoms have been noticed in the monkey excepting an increased irritability, but during fever all of the animals have suffered to a greater or less extent from weakness, loss of appetite, occasional intestinal disturbances, and coughing. No eruption that could be interpreted as being caused by the disease has been observed on the skin or buccal mucosa, and in this respect Mexican typhus fever in the monkey differs from that in man, where a very characteristic petechial eruption is an almost invariable occurrence. Many of our monkeys during sickness showed congestion of the conjunctivae. Also a reddening of the face particularly at the corners of the eyes and the root of the nose was frequently noted, but in our opinion this should not be interpreted as an eruption, inasmuch as normal monkeys under our observation occasionally revealed the same reddening.

No micro-organisms which could be cultivated on the ordinary media have been found in the blood of typhus monkeys in any stage of the disease, although numerous cultures have been made from the heart blood of all animals suspected of having typhus. This result agrees with that reported by all other investigators of the subject and the failure to cultivate organisms thus constitutes a partial control of the fact that the monkey actually has typhus,

[1] In his latest publication Nicolle reports the successful transmission of the disease through nine generations of monkeys without any evidence of a diminution in virulence.

eliminating the possibility of certain other septicemiae which might cause an elevation of temperature.

The disease in the monkey terminates with only moderate abruptness, the temperature taking two or even three days to fall to normal. In this respect it resembles the termination of Mexican typhus in man, which has been repeatedly described as a "lysis" rather than a true "crisis." Recovery has been usually rapid, although certain animals later became cachectic and died from other causes. In only three cases (Monkeys 4, 6, and 22) has the disease ended fatally. The autopsy findings in the first two cases are given on pages 56 and 58. Monkey 22 was inoculated with virus which had been carried by passage through two other monkeys (Nos. 7 and 11). He showed no rise in temperature whatsoever as a result, but sickened perceptibly, becoming progressively weaker until his death, which occurred 14 days after inoculation. Before being made the subject of the experiment he was lively and seemingly in perfect health, and inasmuch as I have seen several cases of typhus in man that terminated fatally without showing any rise in temperature I think it highly probable that he died from typhus. Autopsy was made immediately after death. No definite anatomical changes that could be interpreted as the cause of death were found. Intense cerebral and intestinal congestion was present similar to that seen in previous cases, but as in human typhus the absence of local inflammation or other anatomical change was the more significant feature. Subsequently a monkey (No. 38) was killed on the fifth day of a severe course of fever. Again no distinctive changes were found; no enlargement of the spleen; the liver normal but for slight fatty infiltration; the kidneys showing only slight inflammation; the other organs normal except for great congestions of the vessels of the brain and of the abdominal viscera.

The technic employed in all of these experiments on transmission by the injection of virus was the following: Human blood was obtained from patients at the general hospital. Patients were selected who were typical cases at the height of their febrile courses, that is between the sixth and tenth days of fever. The arm of the patient was scrubbed and the anticubital space cleansed and

disinfected. The median basilic vein was then punctured with a sterile needle, and the required amount of blood extracted by means of sterile syringes or side-necked flasks. These latter were provided with cotton-stoppered aspirating tubes, the needle being attached by a short piece of rubber tubing to the side neck. The flasks contained glass beads, and the blood was immediately defibrinated by shaking. As little time as possible was allowed to pass before making the injections. Usually this amounted to three or four hours, as the distance between the hospital and the laboratory was great. Monkey blood was as a rule aspirated from the heart.

Various quantities of blood were used in different experiments. Where the amount of material was not great the peritoneal route was chosen for injection; in other cases a portion was introduced into the peritoneal cavity and the remainder beneath the skin.

Several flasks, each containing from 50 to 100 c.c. of broth, were inoculated with every specimen of blood used, this serving as a control against the presence of micro-organisms other than those of typhus. In most cases a Widal test was also made.

Before injection the blood was diluted with two or three times its volume of salt solution on theoretical grounds, and because experience with Rocky Mountain spotted fever suggested that dilution of the virus might favor infection. Dilution is known to render less effective specific antibodies which may be destructive to the virus, and there can be little doubt that blood drawn from typhus patients on the eighth day of the disease contains germicidal antibodies, particularly since the disease is one which clinically is known to cause the development of distinct immunity. Dilution may also favor infection through the peritoneal route by affording better conditions for rapid absorption or dissemination of the micro-organisms. In experiments with spotted fever it was occasionally noted that smaller doses of virus would cause infection when larger doses of the same material would not, the injections being intraperitoneal. Small doses, as 0.01 c.c., would be usually diluted with 2 or 3 c.c. of salt solution, whereas larger doses, as 5 c.c., would be diluted to a much less degree or not at all.

The animal experiments on typhus fever were confined to the

Macacus rhesus monkey. This animal is normally healthier, is far less ferocious, and is more easily handled than the capuchin, the sinicus, and other varieties of Macacus. Also it was deemed advisable to restrict the experiments to one species of animal in order that the final results could be better compared with one another, and the rhesus was chosen because it could be more easily obtained than other monkeys. The animal has its disadvantages. It normally runs a rather high temperature, and the variations between the morning and afternoon temperatures of uninfected and apparently healthy monkeys are often considerable. The morning temperatures of healthy controls have habitually been from 0.8 to 1.8 or 2 degrees lower than those of the afternoon. The former is commonly below 101° and may lie between 99° and 100° for several days in succession; whereas in the afternoon (from 3 to 4 o'clock) it commonly is found at some point between 102° and 103° or even 104°. The comparatively cold nights and the warmer days of Mexico City may have had some influence on the more extreme variations. We attempted to eliminate this factor as much as possible by warming the room in which the animals were kept from the hours of 5 P.M. to 9 A.M., the temperature at night being about 15° C. Furthermore from the experience of Nicolle with European typhus the rhesus appears to be less susceptible to typhus than are certain other species. Nevertheless, in spite of these difficulties the results obtained in Mexican typhus with the rhesus are definite and easily interpretable.

Transmission from Man to the Macacus by Injection.

On January 11 a quantity of blood was drawn from the median basilic vein of José Hernandez, a patient in the Hospital General (Mexico City), on the eighth day of his sickness. The fever and condition of the patient were typical for an attack of typhus of this duration, the attack being one of moderate severity. Although the skin was quite dark, the spots, not yet petechial, could be seen over the abdomen, chest, axillary skin, arms, legs, and back, and the conjunctivae were reddened characteristically. The spleen showed little or no enlargement on percussion, and it could not be palpated. After the blood was drawn the patient passed through a typical "crisis," which occupied about three days, and recovered.[1]

Two cubic centimeters of the blood of the patient planted in 50 c.c. of broth

[1] Both the onset and the crisis of the typhus fever of Mexico are said to be less abrupt than in the classical European typhus. This has been contended by prominent Mexican authorities, and has appeared in their literature, for a number of years.

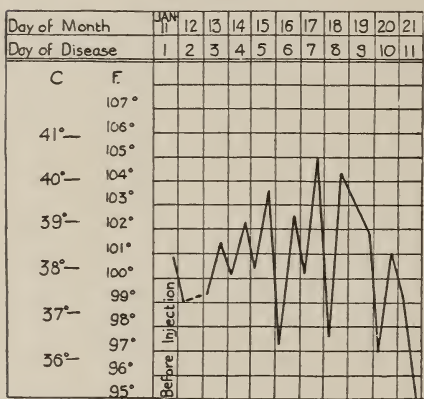
remained free from discoverable micro-organisms, which corresponds with the usual results of cultivation experiments with typhus blood.

Blood inoculation experiment.—Inoculations of 1, 5, and 10 c.c. of defibrinated blood were made respectively into Monkeys 5, 6, and 7. No. 5 died five days after its inoculation, showing a consolidation of the lungs, and since it had had no fever, it was discarded from the experiment. In addition, Monkey 4 received 8 c.c. of the serum from the same blood.

The blood after defibrination stood at room temperature (15° to 20° C.) and in diffuse light for from six to seven hours before injection.

The 5 c.c. of blood which Monkey 6, weighing 2,010 gms., received was diluted to 15 c.c. with sterile physiologic salt solution and the entire amount introduced intraperitoneally. Its temperature on successive days was as recorded on curve No. 1.

CURVE 1.



TEMPERATURE OF MONKEY NO. 6 AFTER INOCULATION.

On January 17, six days after inoculation, the animal ate little and sat "huddled up" with hairs more or less erect. This condition continued and on the 19th there was increased secretion from the conjunctivae and the animal coughed moderately. The illness appeared more severe on the 20th; there was no resistance to manipulation; emaciation; moderate diarrhea. On the 21st, when the temperature became subnormal, the animal was still somewhat responsive until about the middle of the afternoon, when its condition grew rapidly worse, and at 9 o'clock it was moribund.

The autopsy, which was performed at once, showed nothing distinctive and very little that appeared abnormal. The lymph glands generally were moderately enlarged, but were not congested or hemorrhagic. Those of the axilla and groin were the seat of old pigmentation. The lungs were pink, and showed no inflammation or other alteration except for a slight amount of atelectasis at the upper border of the left lower lobe. The pleurae were free from signs of inflammation. Moderate swelling of both the kidneys and liver was present, but they were not degenerated. The spleen was rather firm but not distinctly enlarged. No evidence of infection was found in the peritoneal cavity. The meninges and cerebral cortex were free from congestion, edema, or other signs of inflammation.

A broth flask culture from the heart's blood and agar slants from the viscera remained sterile.

Monkey 7, a male weighing 2,150 gms., received 10 c.c. of the same defibrinated blood, made up to 20 c.c. with salt solution. One-half the quantity was injected intraperitoneally, the other half subcutaneously. Its temperature on successive days is given in curve No. 2.

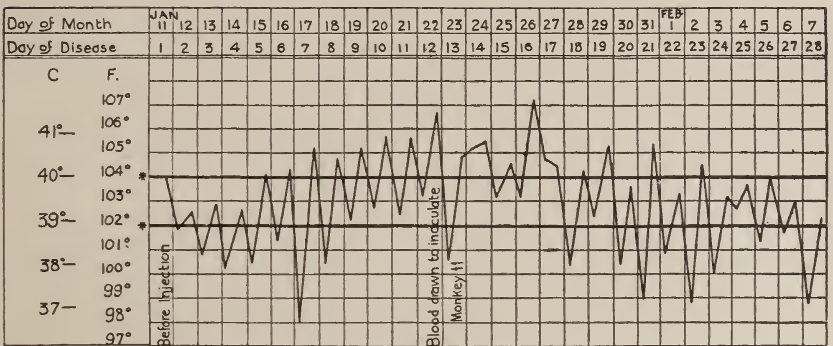
The animal first began to appear sick on the 16th, the second day of fever. On the 17th it made little resistance to manipulation and from this on it appeared dis-

tinctly ill and ate little. It developed no marked diarrhea, although the stools became rather soft. While the temperature became high the animal at no time lost its responsiveness. The conjunctivae were not noticeably reddened, and a distinct eruption could not be identified. After a rather long and severe course, the animal recovered.

Monkey 4, weighing 1,800 gms., received 8 c.c. of serum of the same blood; the serum was obtained by defibrination and centrifugation. This quantity was diluted to 25 c.c. with salt solution, one-half being injected intraperitoneally, the other half subcutaneously. The temperature on successive days was as recorded in curve No. 3.

On the 17th, the first day of distinct fever, the animal, which had hitherto appeared vigorous and healthy, looked sick, unkempt, its hairs stood up, and it "huddled up"

CURVE 2.



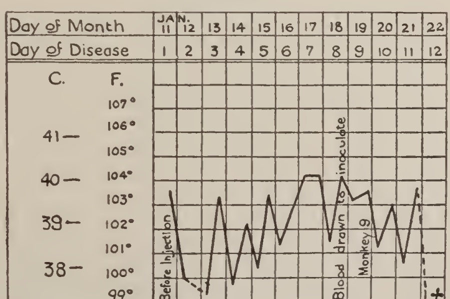
Temperature of Monkey No. 7 after Inoculation.

* The heavy horizontal lines indicate the normal limits of P.M. temperatures. The A.M. temperatures should be lower.

† Remained below 103.6° F. until February 21. Death from peritonitis.

even in the sun. This condition continued, food was refused, and emaciation developed. On the 20th a moderate diarrhea appeared and continued until death. On the afternoon of the 22d it became soporose, and died at about 6 o'clock. An eruption which could be referred positively to the infection, or bearing a resemblance to that of typhus fever, could not be identified. An "eruption" which did appear on the skin of the lower chest and the upper portion of the abdominal skin probably was accidental. On the 18th, the second day of fever, the monkey had been bled from the heart, for cultivation and other experiments. A small amount of blood escaped through the skin when the needle was withdrawn, and at autopsy it was found that some subcutaneous hemorrhage had occurred and that the blood had "diffused" posteriorly in the form of a rather narrow band, fol-

CURVE 3.



Temperature of Monkey No. 4 after Inoculation.

lowing the median line. The "eruption" was roughly median although it extended about an inch beyond the visible limit of the subcutaneous extravasation. In character, it was at first pink, that of the early rose spot, and seemed to disappear on pressure. It appeared two days after the heart had been punctured and on the fourth day of fever. On the following day and subsequently it became darker, more or less cyanotic in color, and could not be effaced by pressure. The spots were rather ill-defined, and appeared to consist of collections of minute punctiform hemorrhages. A similar condition could not be identified on other parts of the body, and in view of the subcutaneous hemorrhage which complicated the situation, it seems probable that the eruption had its source in the latter rather than as a manifestation of typhus fever.

At the autopsy, the lungs were found of a normal pink color; there were no signs of inflammation. On the visceral pleura, particularly of the left lung, were a number of small, circular, dark-red hemorrhages from 0.5 to 1.5 mm. in diameter. The pleural cavities were normal; the heart normal; no inflammation of the valves or pericardium. The liver was apparently somewhat enlarged, pale, as if fatty, but showed little or no congestion; the lobules were well marked; anterior border distinctly rounded. The spleen was about 1.5 cm. longer than that of Monkey 6, and perhaps a few millimeters broader; was distinctly enlarged, bluish-red in color, and of rather firm consistence; contained no hemorrhages.

The kidneys were perhaps a little enlarged and moderately congested; cortex and pyramids of a homogeneous normal color; striations normal; the cortex had a relation to the medulla of about one to one.

The mucous membrane of the colon was much reddened, and perhaps even hemorrhagic; the colon contained a large amount of glairy mucus but no blood or feces. The mucous membrane of the ileum appeared normal, and the ileum contained nothing but a slightly viscous, yellowish fluid; there was a short intussusception, with no inflammatory or obstructive signs of the parts involved. The duodenum contained bile-stained mucus, the mucosa being normal. The stomach contained some undigested banana and mucus.

The lymphatic nodes everywhere seemed more or less enlarged but were not congested or hemorrhagic. Those of the groin and axilla were almost black from some previous pigmentation.

The meninges showed a good deal of congestion and edema, the fluid being perfectly clear. The cerebral cortex appeared normal. Other parts of the central nervous system were not examined.

Cultures, as in the case of No. 6, remained sterile, including those from the meninges.

As a basis for interpreting these experiments, we have for consideration: the existence of an incubation period which was approximately the same in all three animals, and during which they remained healthy; the occurrence of illness and fever followed by the death of two of the animals; the sudden onset of fever and illness, and the rapid defervescence in the animal which recovered; the negative outcome of cultures; and the more or less negative

findings at autopsy, corresponding with the condition in typhus in man.

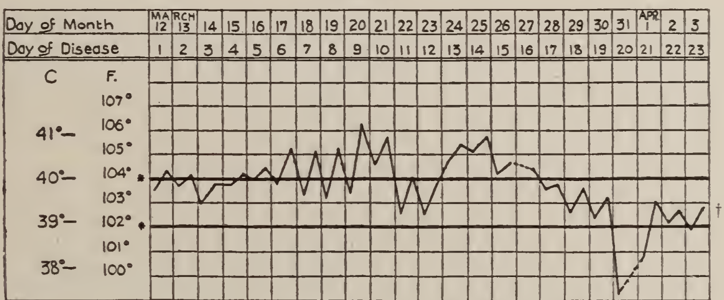
Later investigations have never failed to confirm the results obtained in these early experiments.

Monkey 3 was infected with typhus blood serum. This animal was to all intents and purposes a normal animal, although he had been previously the subject of another experiment (to be described). His temperature curve will be found on p. 67 (curve No. 13).

Monkey 18 was infected with the serum from fresh typhus blood. The temperatures and the details of the inoculation appear on p. 69 (curve No. 15).

Monkey 20, a normal animal, was used to test the virulence of blood employed for other experiments. He thus served as a control for the immunity tests given to Monkeys 1, 3, 13, 25, 24, and 12. All of these animals, together with No. 20, were injected on March 12 with fresh blood taken from a patient (No. 37) in the eighth or ninth day of fever. The blood was defibrinated and diluted with salt solution in the proportions 1 to 2. Each animal received 12 c.c. of the mixture, half subcutaneously

CURVE 4.



TEMPERATURE OF MONKEY NO. 20 AFTER INOCULATION.

* The heavy horizontal lines indicate the normal limits of P.M. temperatures.

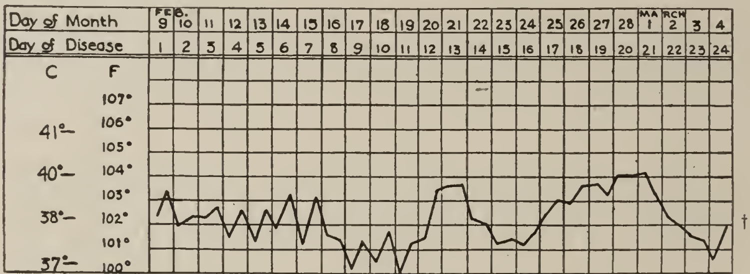
† The temperature of monkey No. 20 had been taken twice daily since February 3 and from that date until March 12 it had remained constantly within the normal limits. During this time the animal was in perfect health.

and half intraperitoneally. The temperature of Monkey 20 on the successive days following the inoculation is plotted on curve No. 4. The course of fever was typical of typhus—an incubation period of five days, a sustained pyrexia of 11 or 12 days, followed by a rapid and complete convalescence. The animal normally ran a rather high evening temperature. Thus on the day of the inoculation 104.3° is recorded, but on this day, in spite of his temperature, he seemed in perfect health. He was well nourished and active, his coat was smooth and glossy, his eyes bright, and his appetite excellent. Quite a different animal was seen on March 21, that is, on the fifth day after the beginning of fever, and from this date until the 27th the monkey was obviously very sick, sitting huddled and unresponsive in his cage, and at times even behaving as if delirious. Broth inoculated with his heart blood on March 25 remained sterile. Recovery was rapid after the subsidence of fever, and by April 9

the animal seemed as well nourished and active as he was before his sickness. He was inoculated again on this date with 4 c.c. of human blood, the virulence of which was controlled by Monkeys 19, 21, and 33. To this second inoculation No. 20 manifested complete immunity, remaining in perfect health until June 16, when observations were discontinued. During this entire period his temperature remained below the limits of possible normal.

Monkey 24 was injected with fresh typhus blood on February 9. After an incubation period of 10 days an elevation of 2° F. is noted (see curve No. 5), and following this date for a period of 10 days the thermal elevation is maintained with the exception of a drop on the 23d and 24th. On the morning of the last day a temperature of 104.1° is recorded, which is at least two degrees higher than the maximum normal limit of *morning* temperatures for the macacus monkey. On the following day

CURVE 5.



TEMPERATURE OF MONKEY NO. 24 AFTER INOCULATION.

† Continued below 102.5° for the following 26 days.

the temperature fell and remained from then on unusually low. In view of the fact that the temperature of this animal before the beginning of the experiment was constantly lower than the average, the thermal elevation, coming as it does 10 days after inoculation and persisting for 10 days, should probably be interpreted as typhus. Cases of mild and abortive typhus occur in man, particularly in children, and the animal in question was one of the youngest of our collection. A bad diarrhea developed about the time of the inoculation, which grew progressively worse. On the 15th he was very weak. On the 21st emaciation and weakness were extreme. On the 25th the conjunctivae were inflamed. The animal was reinoculated on March 12 with blood, the virulence of which was controlled by the injection of monkey No. 20. He resisted this immunity test without the slightest rise in temperature, but continued to grow progressively weaker and died on March 30 as the result of cachexia caused by a persistent diarrhea.

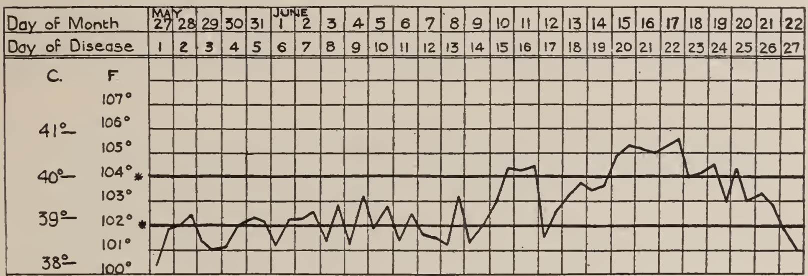
Monkey 43 was the subject of an experiment on the protective power of the serum of convalescents. The serum was found not to protect, and the monkey that was injected with a mixture of serum and virus succumbed 14 days later to a typical course of typhus fever. His temperature is plotted on curve No. 6.

Monkey 44 was inoculated on May 27 to control the virulence of blood being used for other experiments. The animal received 3.5 c.c. of defibrinated blood diluted just before injection with twice its volume of physiologic salt solution. The injection was intraperitoneal. The blood used was drawn at 10:00 A.M. from patient

No. 58 at the general hospital, who was in the seventh or eighth day of a typical typhus fever. The injection was made at 2:00 P.M. The temperatures of the animal appear on curve No. 7.

The reaction exhibited by Monkey 44 was in every respect typical of the course of typhus fever in the macacus. On May 30 the animal had diarrhea and was unusu-

CURVE 6.

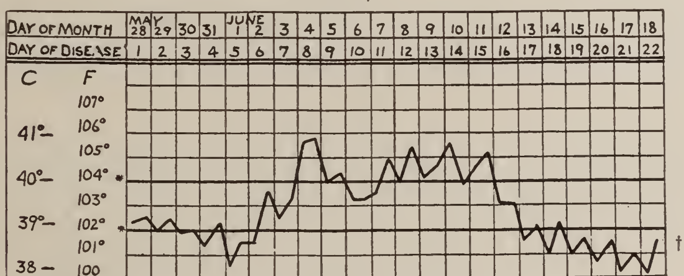


TEMPERATURE OF MONKEY NO. 43 AFTER INOCULATION.

* The heavy horizontal lines indicate the normal limits of P.M. temperatures.

ally irritable. A leukocyte count made on June 1 showed 22,900. By June 5 he was very sick. Broth inoculated from the heart blood on the 5th remained sterile. Smears from the blood revealed the bipolar bacillus described by Ricketts and myself in a previous paper. On this day the leukocytosis equalled 19,650. On June 7 the monkey was still very sick. He sat huddled in his cage, showed little interest in the arrival of his food, and ate nothing. On the 9th he was obviously thinner, and his coat seemed unusually dry and ruffled. The conjunctivae were inflamed. Broth cultures inoculated on the 13th remained sterile. Convalescence was complete and rapid.

CURVE 7.



TEMPERATURE OF MONKEY NO. 44 AFTER INOCULATION.

* The heavy lines indicate the normal limits of P.M. temperatures of the *Macacus rhesus* monkey. A.M. temperatures may be normally much lower.

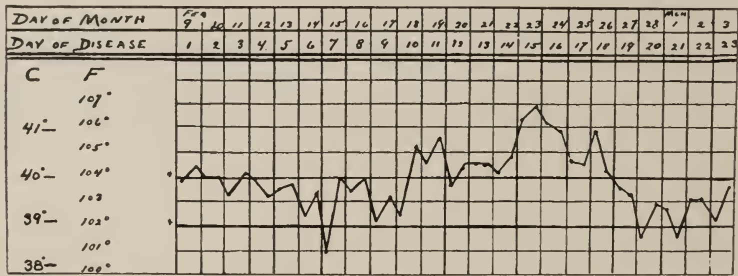
† Remained below 102° until June 21, when observations were discontinued.

THE MINIMUM INFECTIVE DOSE OF TYPHUS BLOOD.

Experiments designed to determine the minimum dose of virulent typhus blood required to infect the macacus monkey were undertaken.

Monkey 25 was inoculated intraperitoneally with 1 c.c. of defibrinated blood obtained from a typhus patient (No. 27) in the eleventh day of fever. The virus was diluted with 5 c.c. of physiologic salt solution before injection. After an incubation period of nine days No. 25 developed a high fever which continued for nine days. On February 21, the first day of fever, the animal began to appear sick, and the following day he was very dejected, sitting huddled in one corner of his cage, shivering, weak, and offering little or no resistance to manipulation. This condition continued until the end of his sickness. His recovery was rapid and complete, and he resisted successfully a subsequent immunity test. The temperatures of this animal during his sickness are recorded in curve No. 8.

CURVE 8.



TEMPERATURE OF MONKEY NO. 25 AFTER INOCULATION.

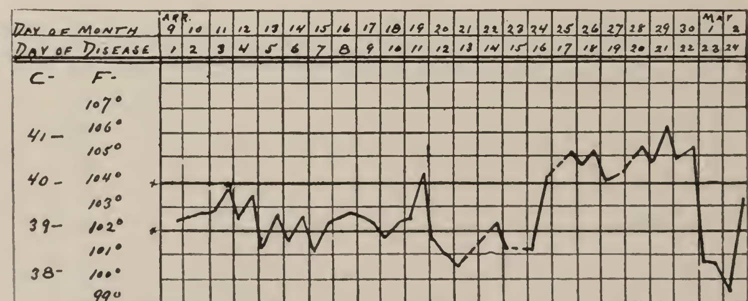
* The heavy horizontal lines at 102° and 104° indicate the normal limits of variation of P.M. temperatures of the species *Macacus rhesus*. The A.M. temperatures may be lower.

† Remained below 103.9° F., 40° C., as long as under observation.

No. 25 was undoubtedly infected by this inoculation of 1 c.c. of typhus blood.

On February 18 Monkey 21 received an intraperitoneal injection of 0.2 c.c. of virulent typhus blood obtained six hours previously from a patient (No. 30) in the sixth day of a typical typhus fever. The blood was defibrinated immediately after it was drawn and was diluted before injection with 2 c.c. of physiologic salt solution. Monkey 21 was apparently not affected by this dose of virus and continued in perfect

CURVE 9.



TEMPERATURE OF MONKEY NO. 21 AFTER SECOND INOCULATION.

* The heavy horizontal lines at 102° and 104° indicate the normal limits of variation of the P.M. temperatures of monkeys of the species *Macacus rhesus*. The A.M. temperatures may be much lower.

† Remained below 103° F., 39.4° C., for the following 30 days.

health and without the slightest elevation of temperature for the following 30 days. A control monkey (No. 18) which had been inoculated with larger quantities of the same virus developed a typical typhus.

No. 21 was later (on April 8) given an immunity test which consisted of the intraperitoneal injection of 3.5 c.c. of virulent typhus blood from Patient 48. To this test he proved susceptible and after an incubation period of 15 days developed a high fever and a typical course of typhus (see curve No. 9).

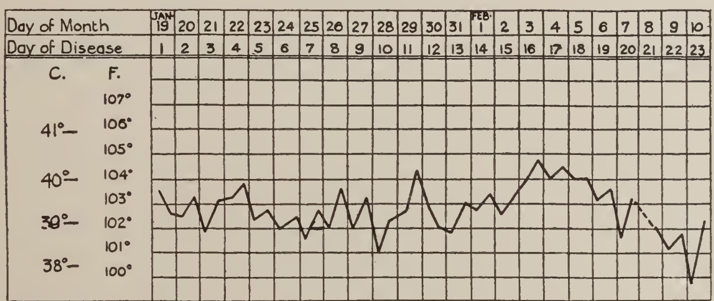
These experiments seem to indicate that the minimum infective dose of blood for the monkey lies between 0.2 c.c. and 1 c.c.

TRANSMISSION FROM MONKEY TO MONKEY BY INJECTION.

Attempts were made to maintain the infection in the monkey by passage from animal to animal.

Monkey 9 was injected intraperitoneally with 5 c.c. of fresh blood drawn from the heart of Monkey 4 on January 18, 1910, that is on the second day of the fever of No. 4 (see curve No. 3). The blood was not diluted. After an incubation period

CURVE 10.



TEMPERATURE OF MONKEY NO. 9 AFTER INOCULATION WITH BLOOD FROM MONKEY NO. 4.

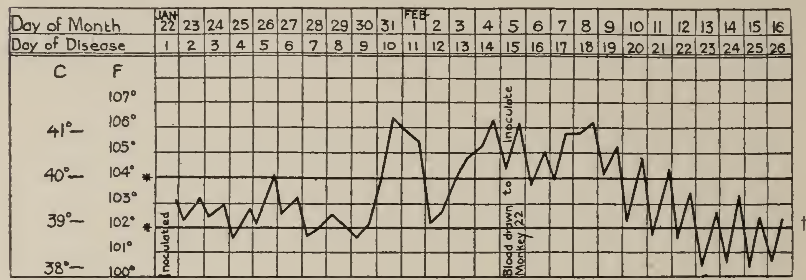
of 10 days No. 9 developed a fever of slight severity which lasted for 9 or 10 days. During this period he was noticeably sick. An immunity test was given 12 days after his recovery. This consisted in the injection of fresh blood from a human typhus patient and was controlled by the reaction obtained with the same virus in Monkey 18 (see curve No. 15). He proved absolutely immune, the temperature remaining below 102.9° F. while under observation.

Monkey 11 was inoculated on January 22 with 5 c.c. of the heart blood of Monkey 7, drawn on the fourth day of the fever of this animal. The blood was diluted with 10 c.c. of salt solution and injected partly intraperitoneally and partly subcutaneously. The change of temperature of the animal taken on successive days following the injection appears on curve No. 11.

On the day of the inoculation Monkey 11 was well and active. By January 31 it was noticed that he was quieter than usual. His face seemed somewhat mottled and was quite red. By February 4 the animal was very sick. Broth inoculated on this date remained sterile. On the 5th 7 c.c. of blood was drawn from the heart,

5 c.c. of which was used to inoculate Monkey 22. Broth cultures made with the remainder remained sterile. On the 7th the animal was still more depressed, sitting huddled in the cage, the coat ruffled and dry. On the 7th, 8th, and 9th he was tied down and lice of Group 9 were fed on his abdominal skin. These lice were employed in experiments detailed a little later. The duration of the fever of Monkey

CURVE 11.



TEMPERATURE OF MONKEY NO. 11 AFTER INOCULATION WITH THE HEART BLOOD OF MONKEY NO. 7.

* The heavy horizontal lines indicate the normal limits of P.M. temperatures.

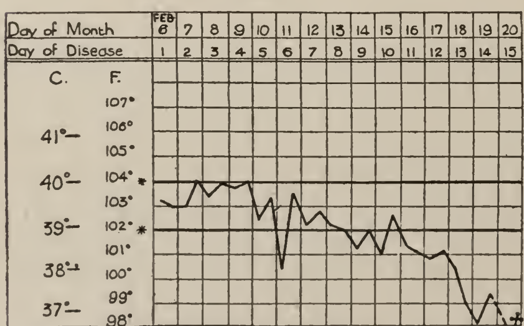
† Remained below 103.4° F. until March 9, when animal was used for an experiment on spotted fever.

11 was 12 days. By February 1 he was recovering rapidly and later resisted an inoculation of human blood which caused in a control (Monkey 21) a typical course of fever. We may therefore conclude that Monkey 11 had been infected with typhus by the injection of blood from Monkey 4.

Monkey 22, inoculated with 5 c.c. of the blood drawn from the heart of No. 11 on February 5, namely, on the sixth day of the fever of that animal, exhibited a peculiar reaction.

On the date of inoculation he appeared to be a perfectly normal animal, strong, healthy, and well nourished, and for the first five days following the injection he seemed to be in good health although his temperature was somewhat elevated, but after this date he sickened rapidly, losing weight progressively, eating poorly. By the 15th his fur was ruffled and dry, he sat huddled and inattentive in his cage, and on the 17th he was so weak that he could hardly support himself on his legs. Now for the first time he had a little diarrhea. His weakness increased and on the morning of the 20th he was found dead, yet during all this time he had no fever, his temperature falling gradually to below normal (see curve No. 12).

CURVE 12.



TEMPERATURE OF MONKEY NO. 22 AFTER INOCULATION WITH BLOOD OF MONKEY NO. 11.

* The heavy horizontal lines indicate the normal limits of P.M. temperatures.

The autopsy findings in this monkey were largely negative, revealing nothing that would account for his death. The body was greatly emaciated. Axillary and inguinal lymph glands were rather large and congested. The abdominal cavity was free from fluid or sign of inflammation. The thoracic cavity contained no fluid, and the lungs and pleura were perfectly normal. The pericardial cavity held a normal amount of clear amber fluid. The heart was unaltered. The liver was enlarged and showed marked fatty changes. The spleen was of normal size, color, and consistency. The kidneys were markedly icteric, somewhat congested, and of normal size. The stomach was distended with gas, but contained no food. The intestines were empty except for bile-stained mucus. Throughout the entire length of the alimentary canal there was no sign of inflammation. The meninges contained a rather large amount of clear fluid, the brain bulging, but there was no cellular exudate. The superficial vessels of the brain were moderately hyperemic. Flasks containing 40 c.c. each of broth were inoculated with 2 c.c. of heart blood drawn at autopsy, and remained sterile. Cultures from the meninges showed a few colonies of staphylococci. The technic of opening the head was crude, however, and as there was no macroscopic evidence of meningitis these growths were judged to be contaminations.

There seems to be nothing to account for the death of Monkey 22 except his injection with typhus blood, and the autopsy rather supports such an interpretation. Afebrile cases of typhus are not uncommon in the literature, and I have myself observed such cases among the patients at the General Hospital of Mexico. One patient who entered the hospital on May 27 in a delirium ran the following evening temperatures for the next nine days: 98.0°, 98.2°, 97.4°, 98.0°, 96.8°, 97.2°, 97.4°, 96.8°, 97.2° F. Death occurred on the following day. The autopsy revealed nothing which could point to any other cause of death than typhus, and the eruption observed during the course of the disease was typical.

In view of the absence of any contrary evidence at the autopsy of Monkey 22 I am inclined to believe that the animal died of typhus fever, and that the atypical course of the disease in his case

is to be attributed to an extreme susceptibility. Unfortunately no attempt was made to continue the passage.

INFECTIVITY OF BLOOD SERUM AND THE FILTERABILITY OF THE VIRUS.

From the above experiments it is evident that the virus of typhus must exist in the blood, and the next question to present itself was whether the virus was simply an intracellular organism or whether it existed free in the blood serum, that is whether blood serum separated from the blood cells would prove infectious. It also became desirable to determine the approximate size of the micro-organism, and these two purposes were served by the following group of experiments.

Blood serum separated by centrifugation was found to be infectious, nearly if not quite as much so as the whole blood, and it was determined that it lost its infectivity on being filtered through a stone filter of fine porosity, a Berkefeld candle.

In the investigation of diseases of unknown etiology filtration experiments of this kind, designed to throw light on the relative size of unknown micro-organisms, have come to be recognized as routine procedures. The filters used are of different porosities. Porcelain filters, known under the names of Chamberland, Reichel, and Pukall, are of rather unequal porosity and will allow particles of about 30 microns to pass, as has been determined by experimentation with suspensions of particles of known size, as, for instance, colloidal gold solutions. The Berkefeld filters used in our experiments are made from siliceous earth and have been found to hold back even the smallest of the known visible bacteria. By the use of this filter one can therefore determine whether or not an unknown virus is of sufficient size to permit of its observation with an oil immersion lens. If it is ultramicroscopic in size it is likely to pass through the pores of the filter and the collected filtrate will still be infectious. If on the other hand it is stopped by the filter it is reasonable to infer that it is large enough to permit of microscopic observation. Accordingly the following experiments were made:

Of the same blood used to inoculate Monkeys 7, 6, and 5, 34 c.c. were centrifugated until the corpuscles occupied approximately the lower three-fifths of the column. The overlying serum was drawn off and replaced by an equal amount of sterile salt solution. The corpuscles were thoroughly mixed or washed with the latter and again centrifugated moderately, after which the overlying fluid was added to the first portion. This was repeated again and the three fluid portions then combined. It seemed probable that through this procedure one might obtain a larger quantity of micro-organisms in the fluid than by resorting to a single more vigorous centrifugation. This seemed better also than to attempt to filter the uncentrifugated blood, which indeed is an almost impossible task with moderate pressures.

It seems sufficiently accurate to consider that the defibrinated blood consisted of about equal parts of serum and corpuscles and that we obtained the equivalent of about 17 c.c. of serum by this washing process.

For the purpose of filtration the serum was diluted to 51 c.c. by means of salt solution, and this quantity was divided into two equal portions, one to be filtered and the other to be injected without filtration.

The first portion was passed through a small Berkefeld candle, and with the threefold dilution it filtered readily. The filter was washed by passing through it an additional 5 c.c. of salt solution, this filtrate being added to the first.

The interval between the drawing of the blood from the patient and the injections was from six to seven hours, as in the preceding experiments.

The total quantity of each portion was approximately 25 c.c., of which one-half was injected intraperitoneally, the other half subcutaneously, after suitable preparation of the skin.

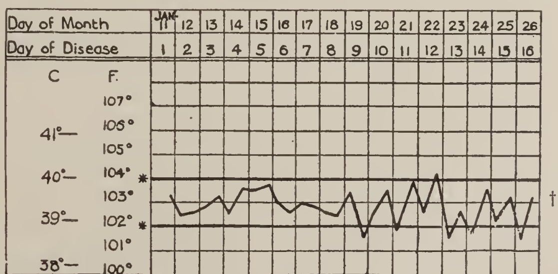
The result of the injection of the unfiltered serum into Monkey 4 was given in an earlier part of this paper, it being our conclusion that the animal became infected with and died of typhus fever.

No. 3, which received the filtered serum, exhibited on successive days the temperatures as recorded on curve No. 13.

In spite of rather high morning temperatures, the animal remained in apparently perfect health, which is in distinct contrast with the course shown by No. 4 (see curve No. 3). As stated previously, the latter, after an incubation period of about six days, developed fever, grew sick, and died eleven days after inoculation, with findings which are in harmony with those of typhus fever.

We may therefore conclude that the virus did not pass through the filter employed, or if it did, that it did not pass through in a quantity sufficient to produce recognizable infection.

CURVE 13.



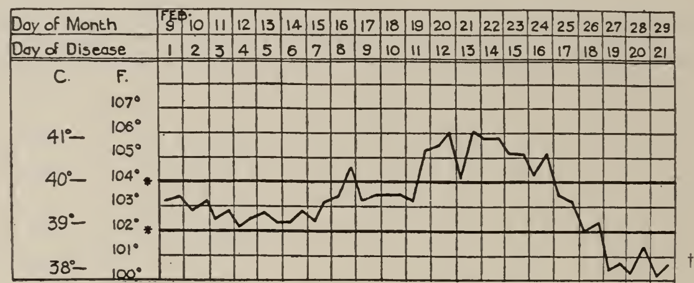
TEMPERATURE OF MONKEY NO. 3 AFTER INOCULATION OF FILTERED SERUM.

* The heavy horizontal lines indicate the normal limits of P.M. temperatures.

† Remained below 103.6° F. until immunity test was made on February 9.

The immunity test confirmed this conclusion. This test, which was given about one month following the "filtration experiment," consisted of the intraperitoneal injection of 7 c.c. of diluted defibrinated blood drawn from a human patient on the tenth or eleventh day of his fever. No. 3, which had tolerated the filtered serum without visible disturbance, showed a course of high fever lasting 11 days, and preceded by an incubation period of seven days, as the result of the immunity test (see curve No. 14).

CURVE 14.



TEMPERATURE OF MONKEY NO. 3 AFTER IMMUNITY TEST.

* The heavy horizontal lines indicate the normal range of P.M. temperatures.
† Remained below 103.6° F. as long as under observation.

Anderson and Goldberger subsequently reported a similar result in a similar experiment.

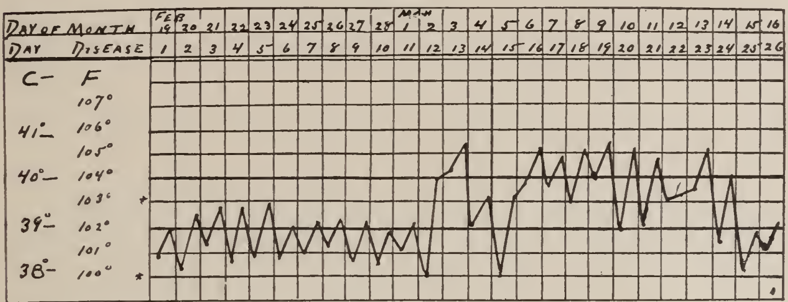
Our experiment was repeated in about the same way, and in the main the earlier results were corroborated. The causal organism of typhus does not pass through the filter in sufficient numbers to provoke a febrile reaction in the monkey.

Two normal monkeys (Nos. 18 and 19) were inoculated with serum from the blood of a typhus patient (No. 30), the former receiving the pure serum, the latter a portion of the same which had previously passed through a Berkefeld candle. The dose of serum in each case was 7.5 c.c., diluted with 22.5 c.c. of physiologic salt solution, one-half of the entire volume being injected subcutaneously and the remainder intraperitoneally. The accompanying temperature curves (Nos. 15 and 16) of the two monkeys indicate the result: No. 18 ran a severe course of typhus, while No. 19 who had received the filtered serum showed at no time during the following 49 days any indication of discomfort.

Thus far the results are the same as those obtained in the previous experiment, but on April 8 an immunity test, consisting of the injection of 4 c.c. of virulent typhus blood, was given to

both of these animals. No. 18 proved immune, as was to be expected, but, whereas before the animal which had received the injection of filtered serum had proved *susceptible* to a subsequent immunity test, No. 19 was found to be immune. The blood used for the test of Nos. 18 and 19 was obtained from a patient (No. 48) who at the time was in the eighth day of fever. The eruption

CURVE 15.



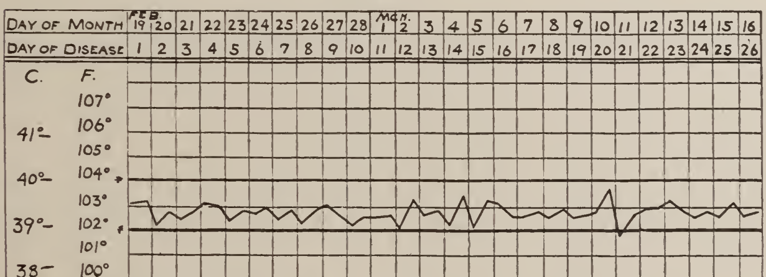
†

TEMPERATURE OF MONKEY NO. 18 AFTER INOCULATION OF UNFILTERED SERUM.

* Normal limits of variation of temperature of monkey No. 18 as long as under observation indicated by heavy horizontal lines.

† Continued below 103° F., 39.4° C., as long as under observation.

CURVE 16.



TEMPERATURE OF MONKEY NO. 19 AFTER INOCULATION OF FILTERED SERUM.

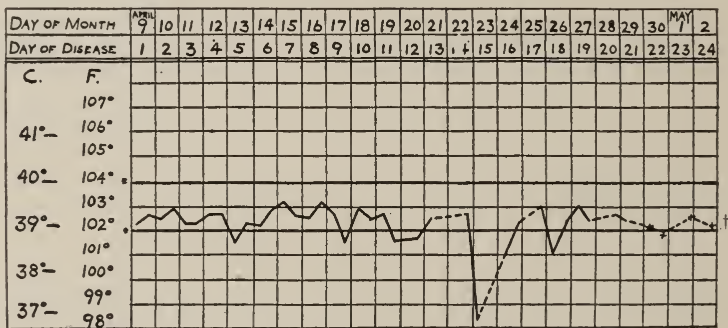
* The heavy horizontal lines at 102° and 104° indicate the normal limits of variation of the P.M. temperatures of monkeys of the species *Macacus rhesus*. A.M. temperatures may be lower.

was profuse and petechial and the subsequent history entirely characteristic of typhus. Other monkeys inoculated with this same material, e.g., monkey No. 21, whose temperature curve is given above, and No. 33, whose history follows, reacted in the usual manner with a distinct febrile reaction.

The refractiveness of monkey No. 19 to this inoculation of

blood may be explained by one or more of several different hypotheses: The animal may have been naturally immune to typhus. If such is the case, however, he is the first normal monkey with which we have had to deal that has shown such an absolute immunity when inoculated with over 1 c.c. of virulent blood. A second possibility is that the animal was immunized by the filtered serum. The filtrations in the two experiments were probably not identical: thus different filters were used and, although both were new and of the same pattern and size, individual filters are subject to variations in porosity; and again the suction pressure was not controlled. Hence it is quite possible that in the second experiment substances

CURVE 17.



TEMPERATURE OF MONKEY NO. 19 AFTER IMMUNITY TEST.

* The heavy horizontal lines at 102° and 104° indicate the normal limits of P.M. temperatures of the *Macacus rhesus* monkey. A.M. temperatures may be lower.

† Remained below 103° F., 39.4° C., for the following 44 days.

filtered through which on the previous occasion had been held back, and that these substances produced the immunization of No. 19.

Such immunization could have been accomplished either by micro-organisms sufficiently small to pass the filter, by fragments of organisms, or by toxins. The serum used on the two occasions came from different patients and these patients were not in the same stage of the disease at the time their blood was drawn. It is possible that the serum of the one was richer in micro-organisms than that of the other, this difference alone accounting for the diverse results obtained in the two experiments. Or the microbe of typhus may pass through several different stages of growth in

a pleomorphic development and, whereas the first serum contained only larger, non-filterable forms, the second had a proportion of minute spores or segments.

On the other hand it is conceivable that certain toxins or fragments of micro-organisms passed the filter and that the amount of these in the first case was sufficient to immunize. The subject deserves more attention since a means of vaccination is suggested which might prove of practical value, and I regret that circumstances made it impossible for me to repeat these particular experiments.[1]

EXPERIMENTAL STUDIES ON INSECT TRANSMISSION.

This investigation included a series of researches bearing on the relation of the body louse to Mexican typhus fever. A preliminary report on the subject appeared in *Jour. Am. Med. Assn.*, April 16, 1910, and at that time in commenting upon the results obtained, emphasis was laid on the following considerations:

The first of these relates to the method of diagnosis of typhus fever in the monkey. As stated, every normal macacus inoculated with a dose of typhus blood equaling 1 c.c. or more has reacted with a well marked course of fever and characteristic clinical symptoms. Furthermore every monkey who has once had typhus whether the attack was severe or mild has proved immune to a later inoculation or immunity test. Reference to the protocols of the experiments with direct inoculation of typhus blood given above will reveal how uniformly all of these animals resisted their immunity tests. It follows therefore that the immunity test constitutes a reliable and accurate method of determining whether or not a course of fever was due to infection with typhus. Such a test consists in a second injection of virulent blood after the subsidence of fever. The virulence of the blood must of course be controlled by the injection of an equal quantity of the same blood into a normal monkey.

The degree of susceptibility of the monkey is another important consideration. As I have shown the macacus is relatively insus-

[1] Nicolle in his latest paper reports that in one instance the serum obtained from infected blood by allowing the clot to separate spontaneously proved immunizing for a *Macacus inuus*. Although his other experiments were negative, he argues that this one positive result is sufficient to demonstrate the filterability of the typhus microbe. This conclusion does not seem to me to be justifiable.

ceptible to typhus and can only be infected by the injection of a large quantity of virus. Also it appears, from the experience of Nicolle, Anderson and Goldberger, and McCampbell, that the infection dies out in the monkey and cannot be maintained by passage.[1]

From these considerations it might appear a priori that the monkey is not sufficiently susceptible for satisfactory results from insect experiments. The question arises, however, as to whether he may not react to the bites of infected insects by an infection of a mild type which will not be accompanied by a high fever, but which might nevertheless be recognized by later giving the animal an immunity test. If he proves immune to a dose of blood of known infectiousness it is to be presumed that he owes his immunity to his previous exposure to the insects.[2]

Such has indeed been our experience. In none of our insect experiments were we able to provoke in the monkey a very characteristic febrile reaction, although slight fever was observed in nearly every case, but when a monkey is exposed to the bites of infected lice he is thereby immunized to typhus fever so that he proves refractive when later injected with virulent blood. The same result has been obtained repeatedly. The animals show some slight indispostion and an insignificant thermal elevation after their exposure to the lice, an abortive attack too mild to permit of positive diagnosis, but in almost all cases they are later found to be immune. Therefore I believe that it may be concluded that the monkeys are infected by the bites of the lice.

Thirteen monkeys have been made the subjects of experiments as to louse transmission. Of these eight were subjected to the bite of lice previously infected by feedings on man (typhus patients). All save three were infected and the results of two of these unsuc-

[1] Nicolle in subsequent experiments, which are reported in a recent publication of the *Annals of the Pasteur Institute* (Vol. XXIV, No. 1, January 25, 1911), has apparently accomplished passage through nine generations. In this series five species of ape were used, three chimpanzees, three sinicus, one cynomologus, one inuus, a second sinicus, and another cynomologus. No diminution in the activity of the virus was observed.

[2] The possibility of the occurrence of mild attacks of infectious diseases which establish immunity cannot be denied, as has been shown in experiments on guinea-pigs with Rocky Mountain spotted fever. In the case of two of our typhus monkeys inoculated by the intraperitoneal injection of blood only a very slight elevation of temperature was obtained, a reaction which we were unwilling to diagnose as typhus fever before we had given immunity tests. In both cases, however (Monkeys 9 and 24), when such tests were given the animals were found to be refractive, while controls (Monkeys 18 and 20) exhibited severe fever.

cessful attempts suggest only that certain biological conditions were not observed, as will be discussed later; the third failure seemed to be due to the use of too few insects. In one case, that of Monkey 39, a decided febrile reaction followed the exposure of the animal to the lice. Two monkeys have been infected by lice which had fed previously on infected monkeys, and two scarification experiments, performed with the intestinal contents of infected lice, resulted in positive transmission.

Monkey 39 was infected by 17 lice, while a negative result was obtained with 10 lice on Monkey 37, and it therefore appears that the minimum number of lice required to infect monkeys lies somewhere between these two figures. The failure to infect with a smaller number may be due to either or both of two possibilities: first, that the monkey is relatively insusceptible as compared to man, and, second, that when any given group of lice are fed on a typhus patient only a small fraction are thereby rendered "infectious," i.e., able to infect a second host. It is probable that if experiments on man could be performed they would reveal that one truly infected louse could alone give the disease to man, and I realize that our results may be criticized on the grounds that the use of such a large number of lice in these experiments does not reproduce the natural condition of infection in man, the transmission obtained being purely mechanical and accidental. This objection is met, first, by an experiment described below which indicates that the young of infected lice although themselves never directly exposed to infection are able to immunize, i.e., infect a second host, and, second, by the results of certain investigations which seem to show that reproduction of the virus actually occurs in the body of the louse. Thus in one of the scarification experiments, the protocols of which follow, a monkey was immunized by the subcutaneous inoculation of six lice which had been infected by three full feedings on typhus patients. The total amount of blood ingested by each louse during these three feedings could not, on the most liberal estimate, have exceeded 0.01 c.c., the total amount of blood ingested by the six lice being, therefore, well within 0.06 c.c., an amount insufficient, even in the case of the most infectious blood, to cause any reaction on the part of the

monkey. In the experiment reported above 0.2 c.c. of blood produced no effect on Monkey 21. Even the 17 lice that infected Monkey 39 would contain in their entire bodies less than this amount of material and the conclusion that the micro-organism actually proliferates in the body of the louse seems justified.

In these experiments the greatest possible precautions were taken to avoid accidental infection of our animals. Monkeys were imported from districts free from typhus and by means of baths and frequent application of insect powder were kept free from all body parasites. Each infected animal was isolated in a separate cage, which was cleaned frequently, and, in order to control the possibility of accidental contagion, normal animals were confined in cages with the diseased. The temperatures of these controls were taken twice daily, and in no case did any one of them develop typhus. All monkeys which were thus exposed proved susceptible to later inoculations, the result tending to disprove contagiousness.

THE TRANSMISSION OF TYPHUS FROM MAN TO MONKEY BY THE BITES OF INFECTED LICE.

Group 3, *Pediculus vestimenti*, was infected as follows: January 5 they fed for thirty minutes on E.S., on the tenth or eleventh day of his sickness;[1] January 6, on J.V., tenth day of sickness; January 7, on C.T., ninth day of sickness; January 8, on the same patient.

On January 9, 45 lice were alive, and 40 fed on Monkey 1 for about an hour; January 10, 334 were alive, and 23 fed on the monkey; January 11 they were not fed; January 12, 19 fed; January 13, 13 fed; January 14, 11 fed; January 15, 9 fed. The feedings were not carried further.

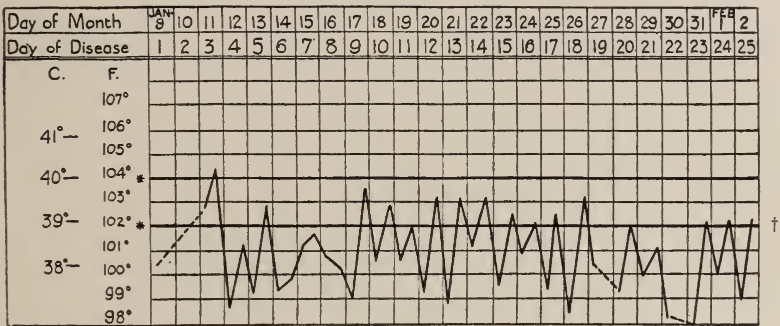
The temperature of the monkey on successive days was as shown in curve No. 18.

It will be noted that in the afternoons of January 17, 20, 21, and 22, the temperature lay between 103.2° and 103.6°, the first rise being eight days after the lice began to feed, or having the 20th in mind, the interval was 10 days. Inasmuch as the animal's temperature was constantly below 102.5° for a period of 31 days later, it is not improbable that the slight elevation referred to represented a mild infection, although no other signs were apparent.

[1] The technic employed in feeding this and nearly all other groups of lice was the following: The anterior cubital skin of the patient's arm, or the abdominal skin of the monkey, in some cases the more vascular face of the monkey, was shaved, cleaned, and dried. The lice lived between feedings in a pill box or test tube which was securely closed. From this receptacle they were taken one by one and placed on the host. They seldom refused to attach themselves promptly to the skin, and, once attached, a large number (50 to 100) could be watched at one time. While the insects fed, the peristalsis of the sucking organ and the stomach gradually filling with blood could be distinctly seen. At the end of an hour or less many would be satisfied and as they detached themselves from the skin they were removed and placed again in confinement. After careful trial of this and other methods, such as keeping the lice beneath inverted bottles, we decided that the danger of losing infected lice was less by this technic than by any other. The lice were carefully counted before and after feeding.

The belief that the animal had suffered from typhus was corroborated by an immunity test consisting of 7 c.c. of defibrinated blood from Patient 27, given February 8. For 19 days thereafter, the morning temperature lay between 99.0° and 102.0°, and in the afternoon between 101.5° and 102.8° (see curve No. 19). This immunity test was controlled by inoculations of 4 c.c. of the same blood[1] into No. 3, and 1 c.c. into No. 25.

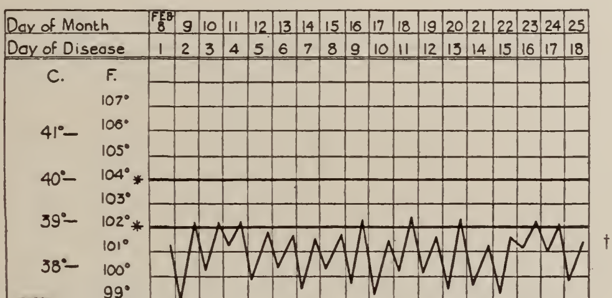
CURVE 18.



TEMPERATURE OF MONKEY NO. 1 AFTER BEING BITTEN BY INFECTED LICE.

* The heavy horizontal lines indicate the normal limits of P.M. temperatures.
† Did not rise above 102.4° F. thereafter.

CURVE 19.



TEMPERATURE OF MONKEY NO. 1 AFTER IMMUNITY TEST.

* The heavy horizontal lines indicate the normal limits of P.M. temperatures.
† Remained low while under observation.

No. 3, after an incubation period of seven days, and No. 25, of nine days, developed fever, which in both animals lasted for ten days, going as high as 106° in the former and 106.8° in the latter (see curves No. 14 and 8).

The immunity of Monkey No. 2 was again tested a month later, March 11, with the same result, whereas Control 20, after an incubation period of five or six days, passed through the usual course of high fever. The first immunity test, however, is

[1] No. 3 had been injected previously with filtered serum without becoming infected; hence this injection constituted an immunity test for No. 3.

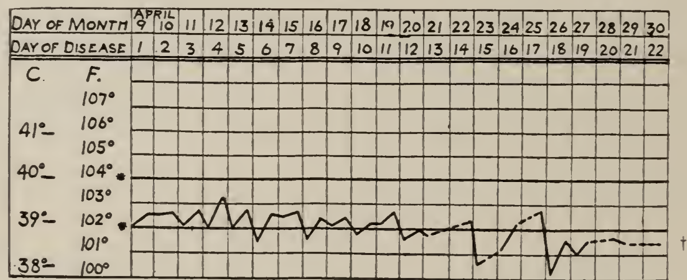
the one which indicates, and it would seem conclusively, that No. 1 was infected by the lice.

Group 11 (*Pediculus vestimenti*) was infected by two full feedings on typhus patient (No. 30) in the seventh and eighth days of his sickness.

On February 21, the day following the last feeding of the group on the infected host, 50 lice were alive and 37 fed on the shaved face of the normal *Macacus rhesus* (Monkey 28) for about one hour. February 22, 45 were alive and 29 or 30 fed on the monkey; February 23, 37 remained alive and 30 fed; February 24, 25 fed; February 25, 23 fed; February 26, 12 fed.

Monkey 28 remained to all appearances in perfect health during the following three weeks. On one day only, March 15, 10 days after the first feeding of the lice, did his temperature rise above 103° F. (39.4° C.), and the temperature of 103.4° F. (39.7° C.) noted on this occasion is well within the normal afternoon variation of the monkey.

CURVE 20.



TEMPERATURE OF MONKEY NO. 28 AFTER IMMUNITY TEST.

* The heavy horizontal lines at 102° and 104° indicate the normal limits of the P.M. temperatures of monkeys of the species *Macacus rhesus*. A.M. temperatures may be lower.

† Remained below 103° F., 39.4° C., for the following 46 days.

On April 8, nearly a month later, he was given an immunity test consisting of the injection of 4 c.c. of blood from a typhus patient (No. 48), recently drawn and defibrinated. To this injection he proved absolutely refractive, continuing in excellent health until June 22, when observations were discontinued. During the entire period his temperature never mounted above 103° F. (39.4° C.), although controls inoculated with the same material developed typhus of moderate severity (see curve No. 20).

This resistance to the immunity test seems to indicate that Monkey 28 was infected by the lice of Group 11.

TRANSMISSION OF TYPHUS TO THE MONKEY BY LICE TAKEN FROM THE GARMENTS OF TYPHUS PATIENTS.

Lice (*Pediculus vestimenti*) were collected on the morning of March 4 from the garments of patient No. 34 on his entrance into the typhus ward of the general hospital. The patient could not tell how long he had been ill, but from his eruption appeared to

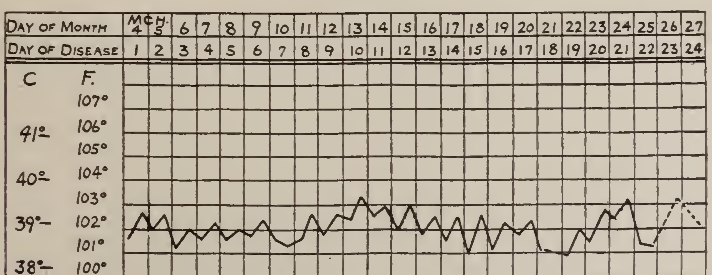
be in the eighth or ninth day of his sickness. He subsequently completed a typical typhus course of moderate severity.

The monkey used for this experiment (No. 17) had been the subject a short time before of a similar experiment, but on that occasion the lice placed upon him apparently all died without feeding. The animal had been washed with green soap, and enough of the soap seems to have remained on the skin to kill the lice. In the second attempt he was clothed in a heavy canvas jacket, which contained a plaited lining of soft wool. The adult lice were placed between the jacket and the skin and the animal confined in a "louse-proof" cage, a cage that had been previously enveloped in muslin to prevent the lice from being forcibly thrown out and that was placed on a square piece of oil-cloth bordered by a strip of eight-inch sticky fly-paper. The method in feeding the lice followed on this occasion was designed for the purpose of raising young lice. In this we were only partly successful.

The lice were placed on the monkey on the afternoon of March 4. On March 8 the jacket was removed and examined; only 12 living lice and 13 dead ones were to be seen, the remainder having probably died and fallen to the bottom of the cage. The live lice had all gorged themselves with blood. Some 200 eggs were also observed, and the jacket was replaced on the animal. On the 14th it was again examined and found to contain no adults, but about a dozen young lice of the second generation. All of these were gorged with blood.

The temperature of the monkey after the first exposure to the lice is given in curve No. 21. It will be noted that a very slight elevation of temperature occurs between March 12 and 15, following an incubation period of nine days since the first exposure of the animal to infection. On March 13 the animal seemed unusually irritable, but at no time was he weaker than usual or otherwise indisposed.

CURVE 21.



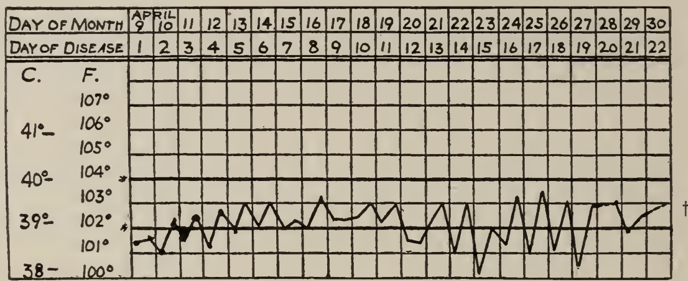
†

Temperature of Monkey No. 17 after Being Bitten by Lice Taken from Clothes of Typhus Patients.

† Remained below 103° F., 39.4° C., for the following 6 days, when observation was discontinued.

On April 8, one month later, Monkey 17 was given an immunity test consisting of 4 c.c. of typhus blood from Patient 48. He resisted the inoculation absolutely, his temperature remaining normal for the following two months. Curve No. 22 records his temperature for the 22 days succeeding his immunity test. Only on two days, April 24 and 25, does the curve rise above 103° F. (39.4° C.), and the slight elevation 103.3° F. (39.6° C.) is not above normal for monkeys. The controls, Monkeys 21 and 33, inoculated with the same blood, ran fevers lasting from six to 10 days and reaching elevations of 106.3° F. (41.3° C.) and 104.9° F. (40.5° C.) respectively.

CURVE 22.



TEMPERATURE OF MONKEY NO. 17 AFTER IMMUNITY TEST.

* The heavy horizontal lines at 102° and 104° indicate the normal limits of the P.M. temperatures of monkeys of the species *Macacus rhesus*. A.M. temperatures may be lower.

† Remained below 103° F., 39.4° C., for the following 46 days.

TRANSMISSION OF TYPHUS FROM MONKEY TO MONKEY BY LICE.

Monkey 7 was infected by the injection of blood from man, as described above.

Group 5, *Pediculus vestimenti*, was infected by three feedings on Monkey 7, on the sixth, seventh, and eighth days of its fever. Thereafter, the lice were fed for eight successive days on Monkey 12, their number gradually decreasing from 81 on the first day to nine on the last day of feeding.

The temperature of No. 12 was irregular and rather high prior to the experiment, although the animal was active and well nourished.

Its temperature continued as in curve No. 23.

A definite course of fever cannot be made out positively. If present, it would appear to lie between January 30 and February 5 or 6, a period of seven or eight days, and the incubation period would be seven or eight days. On February 2 the animal was manifestly ill, in contrast to its former active condition, and this condition continued for four or five days. On February 9, however, it was again active and appeared well. At this time an immunity test was given; the temperature is given in curve No. 24.

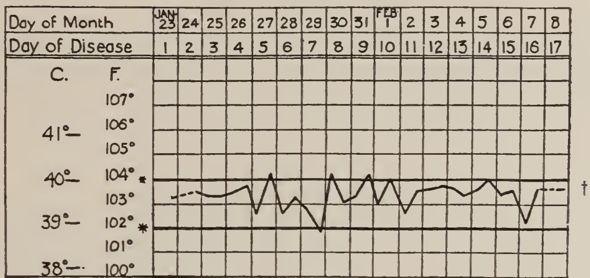
The controls were Nos. 3, 24, and 25, already cited. A second immunity test resulted in the same way, No. 20 (see above) being the control. During neither of the immunity tests did the animal show any sign of illness.

No. 25 is a particularly good control for No. 12, since, like the latter, its temperature was naturally high and irregular. The course of fever was as shown in curve No. 8.

In our opinion, the result justified the conclusion that No. 12 was infected by the lice of Group 5.

Lice of louse Group 10 (*Pediculus vestimenti*) were collected partly from the garments of two *non-typhus* patients at the General Hospital of Mexico City, partly from pupils at a neighboring school, and partly from the clothes of a healthy workman employed temporarily at the laboratory. When obtained the lice were presumably normal. They were infected as follows: From February 12 to 16 they were allowed to feed daily on Monkey 22, which had been inoculated seven days before with blood from Monkey 11, the latter animal being in the sixth day of a typical course of typhus

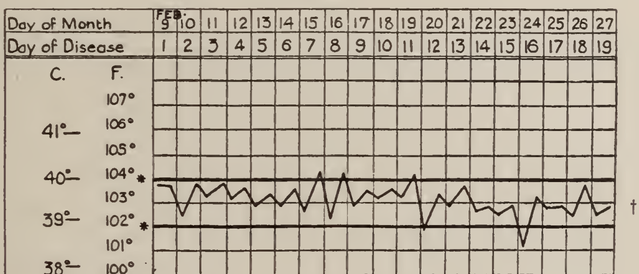
CURVE 23.



TEMPERATURE OF MONKEY NO. 12 AFTER BEING BITTEN BY INFECTED LICE.

* The heavy horizontal lines indicate the normal limits of afternoon temperatures.
† Received immunity test.

CURVE 24.



TEMPERATURE OF MONKEY NO. 12 AFTER IMMUNITY TEST.

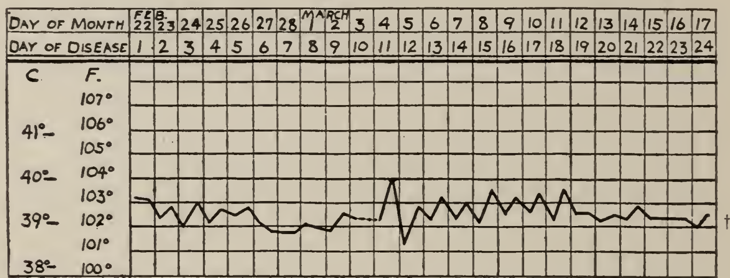
* The heavy horizontal lines indicate the normal limits of afternoon temperatures.
† Continued below 103.9° F. while under observation.

fever at the time the blood was taken. As was mentioned above, this monkey (No. 22) at no time developed fever, but, although normal when inoculated, became progressively thinner and weaker and died fourteen days later, showing no positive anatomical lesions to account for his death excepting intense meningeal congestion, edema, and pronounced hyperemia of the abdominal and subcutaneous vessels, findings highly suggestive of typhus. At the time, however, we were not satisfied with the infection of this animal and hence on February 17 the lice were fed on Monkey 3, which had been inoculated eight days before with blood from a human case of typhus

and was beginning to show an elevation of temperature. No. 3 ran a typical and severe course of typhus. On February 18 the lice were not fed, but on February 19 and 20 they were placed on Monkey 25 during the second and third days of his sickness. This animal had been inoculated with human typhus blood and contracted a severe course of typhus fever of nine days' duration. His history and a curve of his temperature are given on p. 68 (curve No. 14).

After their last feeding on an infected host the lice were allowed to rest for 24 hours and were then placed on the shaved skin of the face of Monkey 29, a normal *Macacus rhesus*. The feedings on this animal were as follows: February 22, 27 fed; February 23, 18 fed; February 24, 15 fed; February 25, 7 fed; February 26, 1 fed.

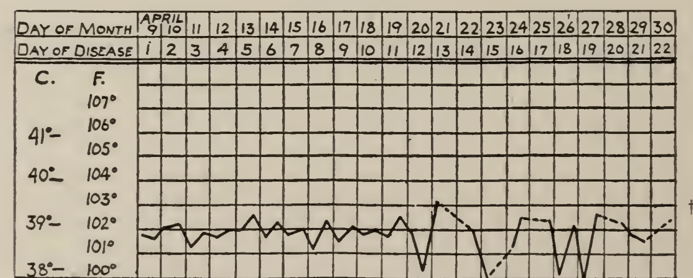
CURVE 25.



TEMPERATURE OF MONKEY NO. 29 AFTER BEING BITTEN BY INFECTED LICE.

† Remained below 103.1° F., 39.5° C., for the following 15 days.

CURVE 26.



TEMPERATURE OF MONKEY NO. 29 AFTER IMMUNITY TEST.

† Remained below 103° F., 39.4° C., for the following 28 days.

The temperatures of Monkey 29 are recorded in curve No. 25. The slight elevation between March 4 and March 11, coming as it does after an incubation period of 10 days, beginning rather abruptly and followed by constant lower temperatures, is suggestive of typhus, but more significant is the fact that the animal resisted absolutely an immunity test given on April 9, one month later. This test consisted in his inoculation with 4 c.c. of defibri-

nated typhus blood from Patient 48. Controls, Monkeys 21 and 33, previously discussed, were injected with the same material and became infected with typical typhus, while Monkey 29 showed absolutely no elevation of temperature or other sign of discomfort (see curve No. 26). I believe that we were justified in concluding that this animal was infected by the lice of Group 10 with a mild attack of typhus.

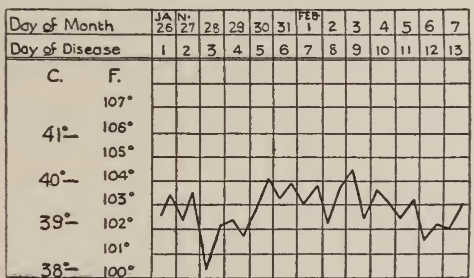
INFECTION OF THE MONKEY BY INOCULATION WITH THE ORGANS OF LICE.

A first experiment, which consisted of the subcutaneous injection of the intestinal contents of infected lice, resulted in death in less than twenty-four hours, from septicemia.

A second experiment was performed as follows: As the lice of Group 5 were feeding on Monkey 12, a small quantity of feces was collected from a number of the lice as it was extruded, and placed in a sterile test-glass. To this material the abdominal contents of three lice were added and the mass was triturated in sterile salt solution. This was done three days after the last feeding of the lice on the infected Monkey 7.

Twelve small incisions, each less than one-eighth inch in length, and extending through the entire depth of the skin, were made in the abdominal skin on Monkey 13. The emulsion of feces and abdominal contents was then instilled into these incisions, which thereafter were massaged by means of a sterile probe. The incisions healed promptly and without suppuration. The temperature of No. 13 was as shown in curve No. 27.

CURVE 27.



†

Temperature of Monkey No. 13 after Infection with Abdominal Contents of Infected Lice.

† Continued low as long as under observation.

As appears on the curve the temperature rose on the fifth day after inoculation and remained above the normal for this animal for five or six days. During this period the animal became passive and was not inclined to run about, although it was not seriously ill at any time.

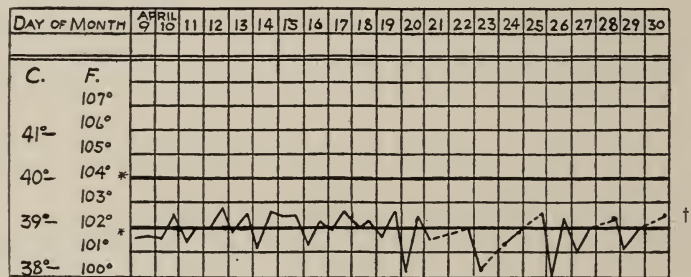
As a consequence of an immunity test given on February 9, the animal showed no febrile reaction whatever and appeared perfectly well, whereas the controls (Nos. 3, 24, and 25) reacted with severe fever, as stated above. A second immunity test, given a month later, gave the same results, the control in this instance being No. 20 (see above).

In our judgment, this experiment proved the existence of the virus of typhus fever in the abdominal contents of the louse for at least three days after feeding on infected blood.

INFECTIVITY OF THE INTESTINAL CONTENTS OF THE LOUSE.

Lice of Group 17 (*Pediculus vestimenti*) were infected by three successive daily feedings on a typhus patient (No. 31) during the eighth, ninth, and tenth days of his sickness. After a rest of 48 hours, during which time they were kept at a temperature of 11 to 12 degrees C., the intestinal contents of six full-grown members of the group were dissected out and introduced beneath the skin of Monkey 32 in the following manner. Care was taken not to rupture the organs during dissection, and until the moment of

CURVE 28.



TEMPERATURE OF MONKEY NO. 32 AFTER IMMUNITY TEST.

* The heavy horizontal lines at 102° and 104° indicate the normal limits of the P.M. temperatures of monkeys of the species *Macacus rhesus*. A.M. temperatures may be lower.

† Remained below 103° F., 39.4° C., for the following 46 days.

injection they were kept intact in a small amount of physiologic salt solution. Twelve incisions, each two to three mm. in length and extending through the entire depth of the dermis, were cut in the abdominal skin of Monkey 32; the six intestinal canals were then ruptured, emulsified in a small amount of salt solution and introduced into these cuts, the material being forced under the skin beyond the edges of the incisions by means of sterile forceps. The wounded surface was then carefully dressed and subsequently healed with little or no suppuration.

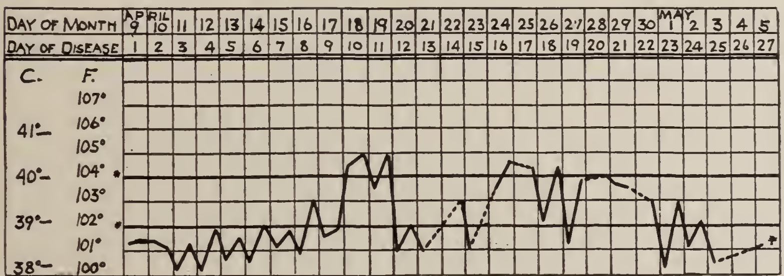
We were unable to observe that Monkey 32 was affected in the least by this operation, for his temperature remained perfectly

normal and he seemed constantly in good health. However, when a later immunity test was given him he was found to be absolutely refractive. This test consisted in the injection of the animal with 4 c.c. of virulent typhus blood, recently drawn from Patient 48. The controls, Monkeys 21 and 33, as mentioned before, which were inoculated with this same material, developed typhus, and the insusceptibility of Monkey 32 is apparently due to his previous inoculation with the intestinal contents of the lice.

AN EXPERIMENT WITH THE HEAD CONTENTS OF INFECTED LICE.

The following experiment was undertaken with a view to testing the infectiousness of the salivary glands of the louse which we thought lay in the head of the insect. We found later that the

CURVE 29.



Temperature of Monkey No. 33 after Immunity Test.

* The heavy horizontal lines at 102° and 104° indicate the normal limits of variation of the P.M. temperatures of monkeys of the species *Macacus rhesus*. A.M. temperatures may be lower.

† Continued beneath 102° F., 38.9° C., until June 22, 1910, at which date observations were discontinued.

head contains little or no glandular material. According to Landois, the salivary glands of *Pediculus pubis*, a member of the same genus as *Pediculus vestimenti* and a very close relative, are confined to the thorax, and we observed glandular structures in the thorax of *P. vestimenti* which resemble the salivary glands of *P. pubis* described by Landois. Powlowski describes, in the head of *P. capitis* and *P. vestimenti*, a minute gland which opens into the junction of the mouth and the pharynx, but the chief salivary glands of *P. vestimenti*, as is the case with most of the insects, would seem to lie in the thorax, and hence an injection of the head contents of infected lice is not a test of the infectiousness of these

glands. As might have been expected, the result of this experiment was negative, and the chief reason for reporting the protocols is that the animal (Monkey 33) by proving susceptible to his immunity test served as a control for the other scarification experiments.

Lice of louse Group 13 (*Pediculus vestimenti*) were infected by a feeding on a typhus patient, No. 32, in the ninth day of his sickness, and by two successive feedings on Patient 33 on the fifth and sixth days respectively of his fever. The group was then allowed to rest for three days.

The head contents of 21 of these lice were dissected out, emulsified in salt solution, and injected hypodermically into Monkey 33. No effect whatsoever was produced in the monkey by the inoculation and a month later, on April 9, he proved susceptible to an immunity test consisting of the injection of 4 c.c. of virulent typhus blood recently drawn from a patient (No. 48). The temperatures of the monkey following his inoculation are recorded on the accompanying curve No. 29. His sickness, although not severe, was quite characteristic of typhus.

A month later Monkey 33 was given a second immunity test and on this occasion proved to be absolutely immune.

DURATION OF THE INFECTIVITY OF THE LOUSE.

The following experiment was performed with the purpose of determining how long lice remain infective after feeding on an infected host, with the hope that thereby some further light might be thrown on the question as to whether transmission by the louse was purely mechanical and accidental or whether it involved the ability of the micro-organism of typhus to live for any length of time within the body of the louse.

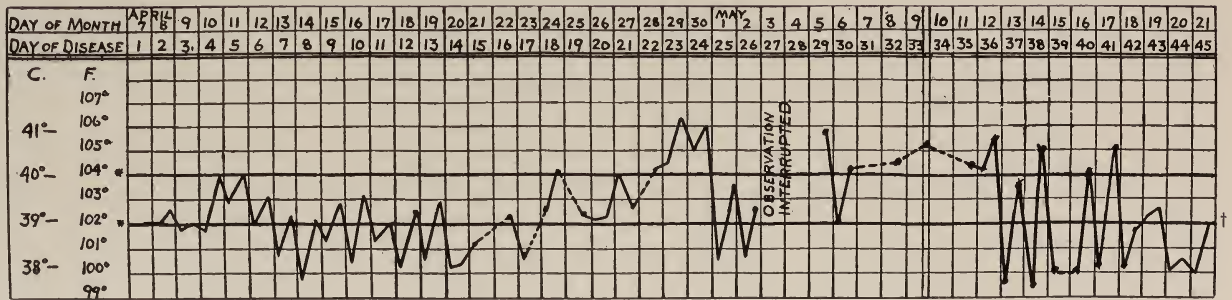
Group 17 (*Pediculus vestimenti*) was infected by feeding for three days, March 29, 30, and 31, on typhus patients (Nos. 41 and 45); on the former during the sixth and seventh days of a severe course of fever. On April 1, 145 of the group were alive and 60 of them were fed on a normal monkey (No. 38). On April 2 and 3, 60 lice were again fed on No. 38, those lice which had not fed on the previous day being chosen from the group.

On April 4, 60 lice were placed on a second normal monkey (No. 36). On April 5 only 58 remained alive; these were all fed on No. 36 and again on April 6, 40 fed on this animal.

On April 7, seven days having elapsed since their last contact with an infected host, 15 lice were fed on Monkey 39. On April 8, these 15 fed again. On April 9 only seven of the group were alive, six feeding on Monkey 39.

In the case of the first two monkeys it was impossible to recognize during the following three weeks either elevation of temperature above normal or other sign of sickness, nor was either of the two protected by the lice against later immunity tests, both reacting with fever of moderate severity to subsequent inoculations of typhus blood. Monkey 39, however, following an incubation period of 17 days began to show irregular elevations of temperature which, on April 29, reached a height of

CURVE 30.



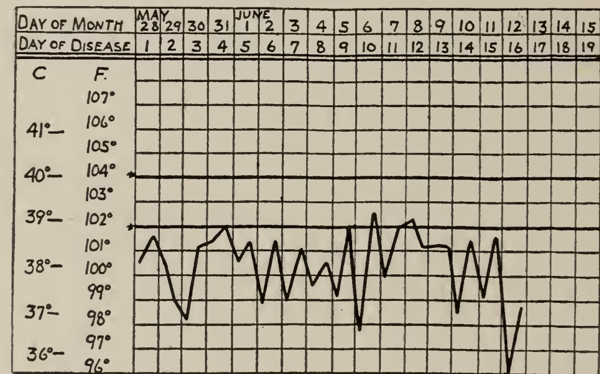
TEMPERATURE OF MONKEY NO. 39 AFTER BEING BITTEN BY INFECTED LICE.

* The heavy lines indicate the normal limits of the P.M. temperatures of the *Macacus rhesus* monkey. A.M. temperatures may be lower.

† Remained below 103.2° F., 39.5° C., as long as under observation.

106.3° F. (41.3° C.). On April 26 the animal was noticeably growing thinner and from then on he sat huddled and inattentive in his cage, his fur ruffled and dry. Broth cultures made from the heart blood on May 30 remained sterile. On the morning of May 1 the temperature dropped to normal and shortly thereafter observations of the animal had to be discontinued. Subsequently the temperatures of this monkey were taken irregularly but from May 5 to 17 they reveal a great irregularity. The nature of the curve during this period, showing as it does large daily variations, is suggestive of some extraneous infection, possibly streptococcic or pneumococcic, but as no cultures were made from the blood at this time the curve is impossible of interpretation (see curve No. 30). The eventual recovery of the animal was very slow, and he remained always thin and unhappy. On May 28, however, his condition seemed slightly improved and on this date he was given an immunity test consisting of 3.5 c.c. of typhus blood from Patient 58 (see curve No. 31). He proved to be absolutely immune, manifesting no febrile reaction, while the control animal (Monkey 44) ran a severe course of fever. Monkey 39 died subsequently, June 15, the post mortem showing acute intestinal and pulmonary tuberculosis.

CURVE 31.



TEMPERATURE OF MONKEY NO. 39 AFTER IMMUNITY TEST.

* The heavy lines indicate the normal limits of P.M. temperatures of the *Macacus rhesus* monkey. A.M. temperatures may be normally much lower.

In view of the resistance shown by No. 39 to the immunity test it seems practically certain that he was immunized by the lice and at least highly probable that the fever occurring between April 23 and May 1 was due to his infection with typhus, certain unsatisfactory conditions in the experiment notwithstanding. That he was sufficiently susceptible to react to an infection by lice with no high fever may be explained by the fact that he was an old monkey, his teeth being hollowed, worn, and decayed. It is known that age plays a large factor in the susceptibility of man to typhus.

Why Monkeys 36 and 38 were not infected or at least immunized

by the lice may likewise be explained by individual variation in susceptibility due to age. They were both quite young and exceptionally healthy animals. It is conceivable also that the infectivity of the louse increases progressively after his infection, due either to the greater proliferation of the organisms, an increase in virulence of the micro-organisms while in the louse, or possibly to their migration to the salivary glands; and hence the result of this experiment points to biological changes of the virus in the louse.[1]

HEREDITARY TRANSMISSION OF THE INFECTIVITY OF THE LOUSE.

This experiment was undertaken with a view to determine whether the young of infected lice were themselves infective, that is, able to give the disease to a host on which they feed. The adult louse contains in its ovaries many mature eggs. These eggs are covered with a compact shell which we thought might prove impermeable to micro-organisms; hence it was decided to rear young lice to maturity on the bodies of typhus patients so that if the eggs were susceptible to infection at any stage of their development they would have every opportunity of being infected within the ovary.

On March 29, 140 adult lice of Group 17 (*Pediculus vestimenti*), 70 males and 70 females, were placed in a stocking on the leg of a typhus patient (No. 41). The stocking was sealed above with adhesive tape to prevent the escape of any of the insects. Two days later 1,000 eggs were found adhering to the fibers of the cloth. The lice were removed and replaced on the patient in a fresh stocking, while the

[1] Attention was called above (p. 40) to the early experiments of Nicolle, Compte, and Conseil on the transmission of typhus by the louse. In Nicolle's latest paper the following results are reported: typhus transmitted from a chimpanzee and a sinicus monkey to four monkeys by lice which had been in contact with their infecting hosts for one to 12 days before, one to six days and five to seven days before, respectively. On the contrary negative results were obtained, that is, neither a febrile reaction nor immunization, on three monkeys bitten by lice which had been in contact with their infecting hosts one to four days before and eight to 12 days before respectively. From this Nicolle concludes that the bite of the louse is not infective before the fifth or sixth day after its contact with its infecting host, and that it is not infective after the seventh day. This observation, it is claimed, supports the theory of the protozoic etiology of typhus fever.

Our experiments, reported above, seem to confirm the first of these observations, namely that the louse is not infective until five or six days have passed since its last contact with its infecting host, but it does not seem justifiable to me to conclude from a single negative experiment on a single animal, as Nicolle does, that the louse is not infective after the seventh day. This observation should first be confirmed by further experiments. Nor do I believe that these observations necessarily support the theory of a protozoic etiology of typhus fever and a life cycle of the micro-organism in the body of the louse, as Nicolle affirms. It is quite as reasonable to consider that a certain lapse of time is necessary for multiplication of the organism to occur in the body of the louse before it is present in sufficient numbers to render the bite of the insect infective.

stocking containing the eggs was put on the patient's other leg. Approximately 800 more eggs were subsequently laid by this generation of lice.

By April 6 many of the eggs began to hatch and by the 15th of the same month about 500 young lice had been collected. These were placed in a fresh stocking which was kept constantly on the leg of a patient in an early stage of fever. For this purpose Patients 47, 49, 52, and 53 were used. Many of the young lice died, but approximately 250 of them reached maturity and laid eggs.

When a sufficiently large number of these eggs of the second generation, presumably infected, had been obtained, all of the adult lice were removed and placed in a new stocking on the same patient. The stocking containing the eggs was then sealed and incubated between the sheet and the mattress of a patient in an early stage of convalescence.

Monkey 42, of the species *Macacus rhesus*, which served for this experiment had been quite recently imported into Mexico from a district free from typhus. As the eggs hatched the young lice were collected and placed on this normal monkey in the following manner: The animal's skin was shaved over the entire abdomen, and a piece of finely woven linen, two by three inches, was tightly secured to the skin by means of a border of two-inch adhesive tape. One edge of the cloth being left unattached, the open end of a tube containing the young lice was inserted beneath this edge, and the lice poured into the pocket formed between the cloth and the skin. The mouth of the pocket was then sealed with tape, and the animal clothed with a heavy canvas jacket in order to prevent interference with the lice.

Thus, on April 28, 50 young lice were placed on the monkey. On April 30, 30 more were added. At this time it was noted that the lice of April 28 had fed, their bodies being gorged with blood. On May 2, 25 additional lice were collected from the stocking and placed on the monkey, whereby in all 105 lice, the offspring of infected lice but themselves never directly infected, were given the opportunity of feeding on Monkey 42.

Unfortunately the temperature of this animal could not be taken regularly during the following three or four weeks, nor was he under very close observation during this period. On May 26, however, he appeared to be in good health. But more important is the fact that he proved resistant to an immunity test. This test, consisting in the intraperitoneal inoculation of 3.5 c.c. of virulent typhus blood from a typhus patient (No. 58), was given on May 27, a month after his exposure to the lice. For the following three weeks Monkey 42 remained in perfect health, although controls inoculated with the same quantity of the same material all contracted typhus of moderate severity, their temperatures maintaining an elevation of 105° to 105.7° F. (40.5° to 41° C.) for a period of 10 or 12 days.

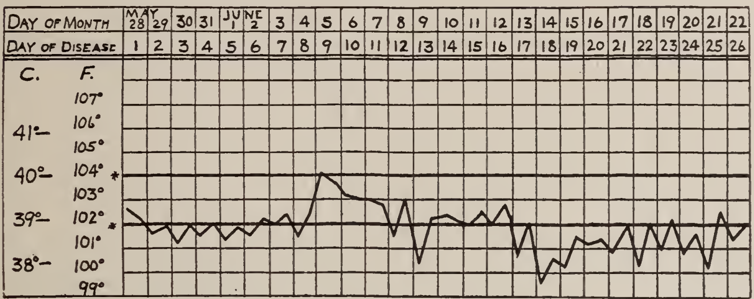
Curve No. 32 shows the temperatures of Monkey 42 on the successive days following the inoculation; curve No. 7 shows those of the control Monkey 44 (see p. 61).

The slight elevation of temperature shown by Monkey 42 on June 5 and June 6 may or may not be an effect of the virus. In any case it is extremely mild and the animal may be said to have been far less susceptible to an injection of typhus blood than the control Monkey 44 or than all normal animals have been found to be.

I appreciate that the result of one experiment does not constitute decisive proof, but the definiteness of the result justifies, in

my opinion, the conclusion that Monkey 42 owed his immunity to his previous infection by the young lice of Group 17, and that hereditary transmission of the infectivity of the louse is established to the extent of reasonable probability.

CURVE 32.



Temperature of Monkey No. 42 after Immunity Test.

* The heavy lines indicate the normal limits of P.M. temperatures of the *Macacus rhesus* monkey. The A.M temperatures may be much lower.

INFECTIVITY OF THE FLEA AND THE BEDBUG.

I have referred on a previous page to certain theoretical considerations which are opposed to the theory that the bedbug and the flea are influential in spreading typhus. It is impossible to explain certain epidemiological characteristics of the disease by assuming that either of these insects is the sole agent in carrying the infection and yet either or both of them may play a rôle subsidiary to that of the louse, and under certain circumstances act as carrier. In order to throw further light upon this question the following experiments were devised:

Infectivity of the Bedbug.—A group of about 50 bedbugs was fed on three successive days on typhus patients at the general hospital. The bugs were confined beneath a wide-mouthed glass bottle inverted over the skin of the patient. In this way 10 or 12 could be handled at one time. All were given the opportunity of gorging themselves with blood.

On April 2, two days after their last feeding on a typhus-stricken host, they were placed on the shaven abdomen of a monkey (No. 38) and allowed to feed. On April 3 they were again placed on the monkey. On April 4 they were not fed. On April 5, 28 bugs fed well on the animal; April 6, 16 bugs fed lightly; April 7, 17 bugs fed lightly; April 12, 29 bugs fed well, gorging themselves with blood.

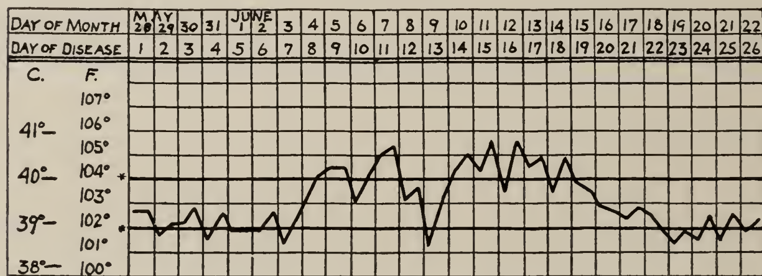
The animal showed absolutely no rise in temperature and continued in excellent health during the following 32 days. Unfortunately he died from an accident before

an immunity test could be given him, and hence the experiment cannot be taken as proof of the noninfectivity of the bedbug. The possibility that the bedbug is better able to transmit typhus than the louse does, however, seem to be eliminated insomuch as both the period of feeding on the infected host and the period of feeding on the monkey were considerably in excess of the feedings of the louse in certain of our experiments.

Infectivity of the Flea.—Human fleas of Flea-Group 2 were infected on typhus patients at the general hospital in the following manner: One or two fleas were confined in each of several long and narrow tubes. These tubes were made of 6 mm. glass tubing, were sealed at one end, and cut sufficiently long (20 cms.) to prevent the escape of the flea by jumping. For this purpose also they were slightly bent in the middle. The fleas were fed by inverting this tube over the skin of the patient.

Subsequent to their last feeding the fleas were allowed to rest for about 60 hours. The entire bodies of 10 of the group were then emulsified in physiologic salt solution

CURVE 33.



TEMPERATURE OF MONKEY NO. 41 AFTER IMMUNITY TEST.

* The heavy horizontal lines indicate the normal limits of the P.M. temperatures of the *Macacus rhesus* monkey. A.M. temperatures may be lower.

and rubbed into scarifications in the abdominal skin of a normal monkey (No. 41), the technic employed in this procedure being the same as that used in the scarification experiment performed with the intestinal contents of lice and described above. The wounds healed with but little suppuration. The animal's temperature was taken daily for the following 34 days and during this time he remained in perfect health.

On May 27 this monkey was given an immunity test, receiving an inoculation of 3.5 c.c. of typhus blood from Patient 58. After an incubation period of seven days he began to run a fever which lasted for 12 days, his temperature being recorded in curve No. 33. On June 4, a leukocyte count was made which showed 30,350. The animal was very irritable and his coat dry and ruffled. On June 5, he had diarrhea, huddled in his cage, and was less active in resisting manipulation and until the 14th of the month he seemed very sick. His subsequent recovery was rapid and complete.

In brief, No. 41 had not been infected by the fleas, as is shown by the fact that he was not immune to a subsequent inoculation of typhus blood.

These results seem to strengthen our position as to the unimportance of the flea and the bedbug in the transmission of Mexican typhus fever.

SUMMARY OF EXPERIMENTAL OBSERVATIONS ON TYPHUS FEVER.

1. A limited number of observations are on record of successful infection of human beings by inoculation of blood from typhus patients.

2. Various species of ape have been found to be susceptible to typhus fever. None of the other laboratory animals thus far tested have been susceptible.

3. In our personal investigations all normal monkeys of the species *Macacus rhesus* have been successfully infected by the inoculation of 1 c.c. or more of virulent typhus blood from man. The blood should be drawn not later than the tenth day of fever and should be diluted with physiologic salt solution before injection. The minimum infective dose lies between 0.2 c.c. and 1 c.c. of defibrinated blood.

4. The course of typhus fever in the macacus after blood inoculation is sufficiently characteristic to enable positive diagnosis and accurate interpretation. The disease runs a course similar to that observed in man. After an incubation period commonly lasting from six to 10 days there occurs a high febrile reaction which continues from eight to 15 days. The long incubation period, the absence of cultivable organisms in the blood and of anatomical lesions are characteristic.

A general leukocytosis is observed quite frequently in the monkey, as in man, and a relative increase in the number of large mononuclears is a constant finding. The temperature falls by lysis, taking two or three days to return to normal. Convalescence is as a rule rapid and complete. One attack of the disease confers absolute immunity, and an immunity test may therefore be relied on to illuminate the diagnosis of doubtful cases in monkeys.

5. We have succeeded in carrying the disease in the monkey through three passages.

6. The serum from the blood of typhus patients is infective.

7. The virus of typhus is apparently non-filterable, blood serum losing its infectivity on passing through a Berkefeld filter. In one experiment, however, a monkey was seemingly vaccinated by an inoculation of filtered blood serum. This result would seem to

indicate that toxins, bacterial fragments, or micro-organisms sufficient to immunize may pass through the pores of a filter.

8. Typhus has been transmitted to monkeys by the *bite* of lice in seven out of 10 experiments. In addition two monkeys were infected with typhus by the introduction into cutaneous scarifications of the intestinal contents of infected lice.

9. The minimum number of lice found necessary to infect a monkey was 17.

10. A monkey was infected and immunized by the subcutaneous introduction of the intestinal contents of six adult lice. The material thus injected proved considerably more infectious than an equal amount of typhus blood, for it has been shown by experiment that more than 0.2 c.c. of blood are required to immunize a monkey, whereas, by the most liberal estimate, these six adult lice did not ingest more than 0.06 c.c. of blood during their three feedings on a typhus host. The conclusion is indicated that the infectiousness of the virus is enhanced within the louse. This may occur by multiplication of the causal organism, or by an increase in its virulence, or by both.

11. In the one experiment attempted, a monkey was immunized, i.e., infected, by the bite of the young of infected lice. As these lice of the second generation had never been themselves directly exposed to infection, hereditary transmission of the infectivity of the louse is indicated.

12. Experiments performed with the bedbug and the flea failed to give any evidence that either of these insects can transmit typhus and support theoretical objections to their consideration as carriers of the disease.

VI.

PROPHYLAXIS.

It seems appropriate to conclude a paper on the transmission of a disease with a discussion of the prophylactic measures which are indicated by the conclusions reached in the course of the study. In this discussion I wish to confine my remarks to the problem of the control and elimination of typhus fever in Mexico City where I am personally familiar with the conditions which favor its con-

tinuation, although I have no doubt that in general my remarks will apply equally well to other cities. I feel firmly convinced that the institution and strict enforcement of the following hygienic measures in Mexico would result in a great reduction in the death rate from typhus, in the control of the epidemic exacerbations which now occur annually in that city, and ultimately in the absolute extinction of the disease.

There is no evidence leading to the suspicion that typhus is ever acquired through the alimentary canal, either in drinking water or as a contamination of food, and measures designed to purify the food and drink of the community will have no direct effect on the amount of typhus fever. In clean surroundings typhus is not "catching," and the disease does not belong to the category of infections commonly spoken of as contagious. Its reputed "contagiousness" has been shown to be entirely dependent upon the presence of infected vermin. Three insects (flea, bedbug, and louse) are open to the suspicion of being typhus carriers, and in the case of the louse sufficient evidence has been adduced to prove that this insect is the chief agent in the transmission of typhus fever.

Prophylaxis should therefore be directed toward limiting the activity first of the louse and then of the bedbug and the flea. Such prophylaxis should contemplate: (1) the general destruction of lice wherever or by whatever means this is possible; (2) the extermination of all insects, fleas, bugs, or lice found on the bodies, clothing, or bedding of persons suspected of having typhus or of the contacts of such typhus suspects; (3) the adoption by individuals exposed to the disease of precautions to minimize the danger of their being bitten by infected vermin.

1. *The general destruction of lice.*—A problem similar to that so successfully met in the campaign against yellow fever confronts us, but the task of exterminating lice will probably be found to be more difficult than was the elimination of the yellow fever mosquitoes. It will even be considered by some as visionary to hope to eliminate lice from the poor population of the Mexican plateau. The harboring of body vermin by them is regarded as perfectly normal and natural, and the antipathy which the Mexican Indian

exhibits to water and the bath can only be overcome by a long and painstaking process of education. The poverty and misery of the great mass of the poor increase the difficulty of inspiring in them any inclination for cleanliness. Nevertheless, despite the discouraging outlook, such education should be undertaken, and I feel that a great deal would be accomplished simply by giving them the opportunity of keeping clean. Public baths and free wash-houses for the lavage of clothes should be erected. The squalor and filth of the poor is chiefly due to the fact that they have no water. For six months of the year the ditches around the city are dry, and in certain districts water must be bought from "aguadores," men who cart it about the streets, at a price which prohibits its use for washing. The fact that the people do make use of the opportunity to clean their clothes when the ditches contain water in the rainy season encourages me to believe that they would use the wash-houses were they provided.

In the public baths there should be facilities for the sterilization of the clothes of the bathers. A short exposure to steam would probably suffice to kill all the lice. In any case care must be taken to prevent promiscuous interchange of parasites between the divested clothing of various individuals.

The establishment of public wash-houses for clothes is in my opinion of greater importance than the baths. In these wash-houses there should be provided tubs for the use of women who bring their washing, and if practicable a general sterilization of all the soiled clothing should be performed. Such sterilization would be best accomplished by heated steam.

In that part of the population, a not inconsiderable proportion, which is under the immediate supervision of the government, personal cleanliness and *absolute* freedom from body parasites may be enforced. I speak of the inmates of hospitals, orphan and correctional schools, and institutions for the poor and incapacitated, the prisons, and army barracks. Mention has been made of the importance of Belem as a distributing center for typhus. Its inmates come from all sections of the city and return to their respective sections when they leave the prison. They have abundant opportunity to pick up infected lice while in the prison

and they disseminate the infection throughout the town. The enforced cleanliness of the prisoners would do away with this condition. The efficacy of a cleanly and sanitary prison administration in eliminating typhus is seen in the case of the Mexican penitentiary. Typhus has on numerous occasions raged in the city around the walls of the penitentiary, but owing to the enforced disinfection of all entrants and the strict cleanliness of the inmates no case of the disease has ever occurred within the walls of that institution.

The presence of lice on persons in the wards of hospitals is inexcusable and may easily be avoided by the bathing and disinfection of the bodies and clothing of all entrants before their admission to the hospital wards. Visitors to the sick should be excluded from the hospital if from their general appearance of uncleanliness it is suspected that they harbor lice. Other insects such as bedbugs should be exterminated whenever they are seen in hospitals and other institutions, and in all of these institutions frequent bathing and the use of clean clothes should be enforced. The harboring of lice by soldiers in the army should be made a punishable offense, and the army barracks be kept free from vermin. Finally, the cleanliness of all the pupils in the public schools should be insisted on. The parents of children found harboring lice should be amenable to fine. The spread of typhus from the schools, which are distributing centers of no small importance, would thus be limited. Prisons, hospitals, charitable institutions, armies, and army barracks, and finally schools, all of which places are now considered to be distributing centers for typhus, could thus be rendered harmless, and the disease would be confined to isolated sections of the city, where it could be fought case by case.

2. *The extermination of insects found on typhus patients or on the contacts of such patients.*—Every case of typhus fever arising in the city should immediately be reported to the department of health. Such cases should be visited promptly by sanitary inspectors, and unless the house and surroundings of the patient are clean and above the suspicion of being infested with vermin, the patient and all of his contacts should be removed to the typhus hospital. If the case occurs in the family of people of cleanly habits, where the

presence of body lice and bedbugs can be excluded, there should be no objection to the treatment of the patient at his home. In this case the house should be quarantined and kept under the strict observation of sanitary inspectors.

At the hospital a separate entrance pavilion should be provided for the reception of typhus patients and their contacts. Here the patient should be stripped and bathed before he is admitted to the wards. The contacts should likewise be bathed and their clothes disinfected. Heat disinfection (steam) will probably recommend itself for the sterilization of the clothing.

In the meantime the house or rooms from which the patient has been removed are to be disinfected, all rugs, bedding, hangings, etc., to be taken to a disinfecting station. The house should then be fumigated with sulphur, the walls repapered or lime washed, and the woodwork and floors washed with soap and disinfectants.

The contacts should be kept at the entrance pavilion of the hospital and provided for until the disinfection of their home has been completed, when they may be allowed to return. After their disinfection they may be considered as harmless and permitted to resume their occupations. They should, however, be kept under observation by the health department for a period of 21 days for fear that some of them were infected before and may develop fever. The incubation period of typhus in man is usually estimated at from seven to 14 days (Parkes) and probably does not exceed 21 days.

3. *Precautions designed to minimize the danger of those exposed to infection.*—So far as is possible typhus immunes should be employed in those occupations which constantly expose to infection, such as the reception at the hospital of typhus patients, their disinfection, and the cleansing of the clothing of patients and their contacts. Sanitary inspectors whose duty it is to fumigate the rooms occupied by patients are unduly exposed and should also be chosen from among immunes. The nurses and attendants in typhus wards should be immune, although these are in relatively little danger if the wards and patients are kept scrupulously clean and free from vermin. Visitors should be excluded from the typhus wards lest

they bring in insects and thus favor the spread of typhus from the patients to such nurses or physicians as are susceptible.

Other individuals, physicians, and students whose duties take them in contact with typhus patients, particularly if these are visited in filthy, vermin-infested surroundings, should observe the following precautions: Superfluous clothing which is liable to brush against the furniture, the bedding, or the clothing of the patient, or which sweeps the floor, is to be discarded. The skirts of nurses should be sufficiently short to be out of danger of touching the floor. Trousers are to be rolled above the shoe tops and coats should be removed. Loose gowns, besides being valueless as far as protecting against the contagium of typhus is concerned, are actually dangerous, as they sweep up any lice that happen to be within a wide radius. The sleeves should be rolled well above the elbows, so that occasional vermin which accidentally crawl onto the hands may be more readily discovered before they find concealment in the clothing. Eucalyptus oil is believed to be unpleasant to insects, and Hay recommends smearing neck, wrists, ankles, and shoe tops with a solution of eucalyptus in olive oil. Finally, frequent change of clothing is advised, and minute attention should be paid to personal cleanliness.

In conclusion I should like to add a word concerning the quarantine against typhus at the United States border. The northern states of Mexico—Nuevo Leon, Coahuila, Chihuahua, and Sonora—which border the Rio Grande are free from typhus for the same reason that the coast states are spared, namely because the greater heat of these regions is incompatible with the transmission of the disease (see pp. 42–43), and it may be presumed on the same ground that Texas, New Mexico, and Arizona incur no serious danger from the importation of typhus. Their climate is also too warm for typhus fever. The same is not true of our more northern states, whose cooler climatic conditions more nearly approach those of the Mexican central plateau. Body lice are far from uncommon among certain classes of our larger northern cities, and other predisposing factors—crowding, squalor, misery, hunger, and want—are never absent in the poorer districts. Should these conditions

become aggravated by industrial unrest, or in the emergency of the outbreak of war, such districts if infected might easily become centers of typhus. It is to our interest that they have no opportunity of becoming infected, and this can only be assured by preventing immigration of *lice-carrying* individuals from other typhus centers.

All patients suffering from typhus fever as well as their contacts should of course be quarantined at the border, as is done now. But more important than this is the necessity of fumigating the clothes and baggage of such immigrants as from their appearance may be suspected of harboring body lice.

At present the immigrating Mexican laborer comes chiefly from the border states and is therefore not apt to harbor *infected* lice, furthermore he rarely travels farther north than Texas or Arizona, but let there be any considerable migration from such hotbeds of typhus as Mexico City or San Luis Potósi directly to our northern cities without the quarantine regulations suggested, and typhus fever, now unknown in the United States, would in all probability gain a foothold.

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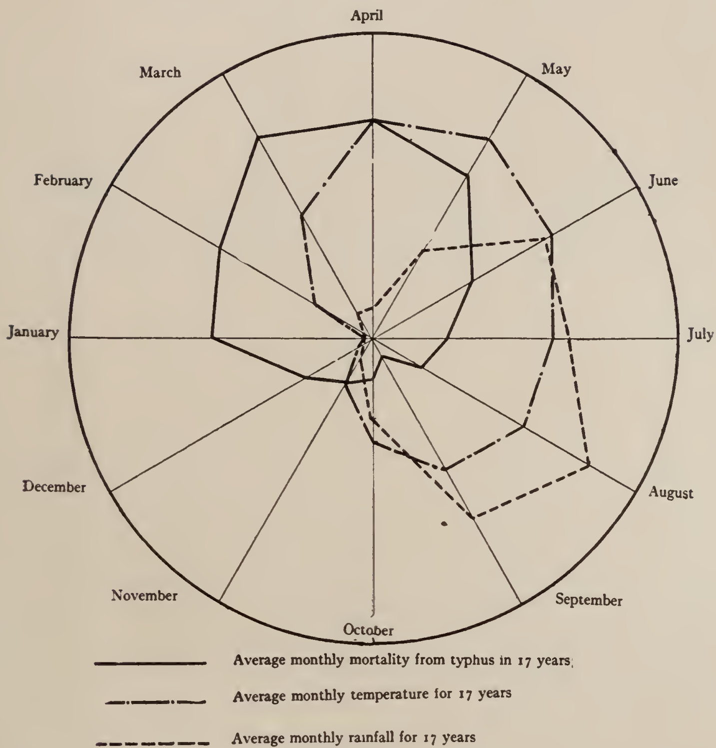
CHART I.

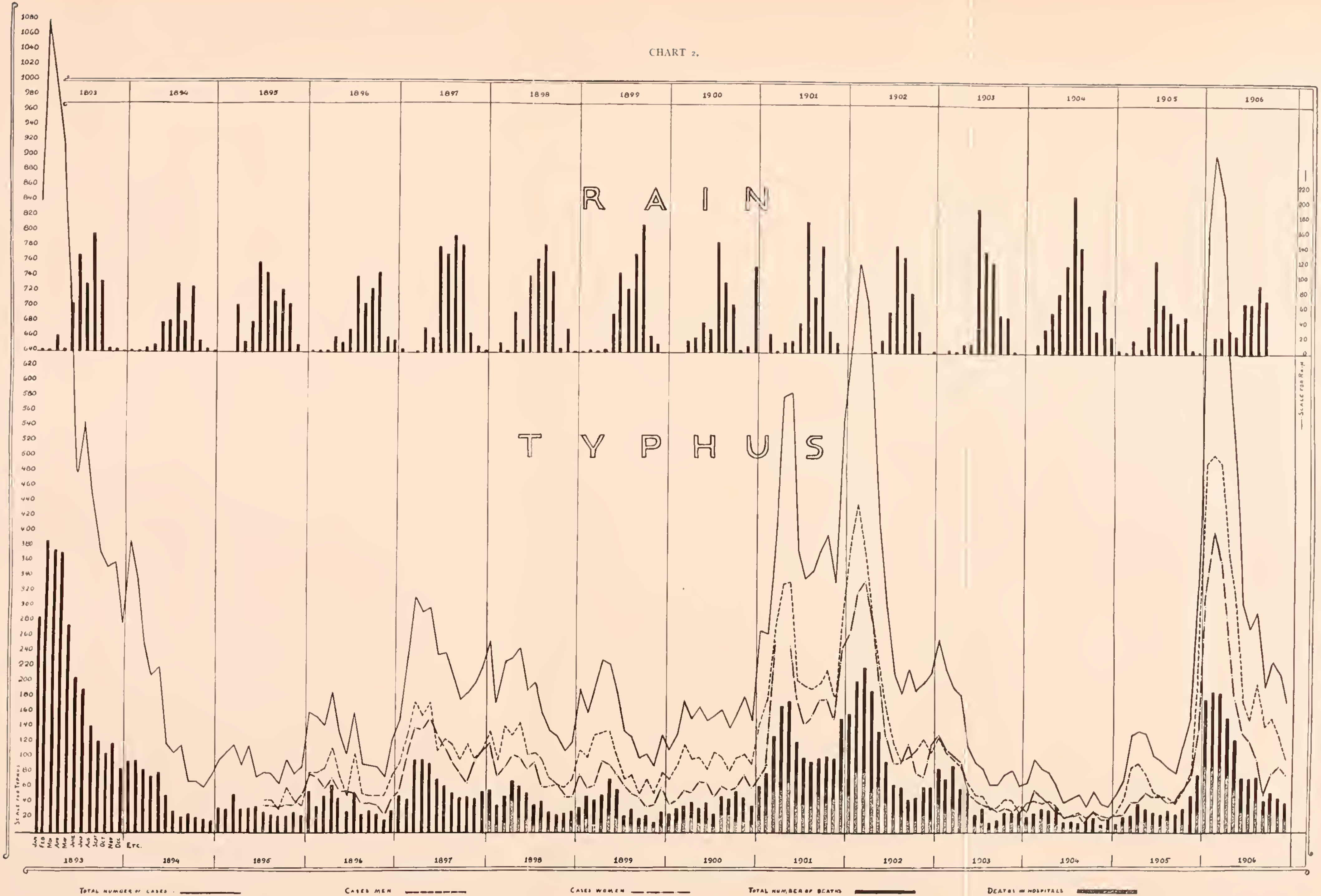
TYPHUS TEMPERATURE AND RAINFALL IN MEXICO CITY.



(After José Terrés, *Etiologia del Tabardillo*)

0.5 centimeter of radius = 10 deaths beginning with 30 at the center.
= 1 degree Centigrade of temperature beginning with 12.
= 20 mm. of rain.

CHART 2.



Drawn from chart in possession of the Consijo Superior de Salubridad of Mexico.

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ON THE LOCAL PRODUCTION OF ANTIBODIES.*

LUDVIG HEKTOEN.

(From the Memorial Institute for Infectious Diseases, Chicago, Ill.)

Whether the power to produce antibodies is widely distributed among the cells of the body or whether it is limited more or less strictly to certain cells or tissues has not been determined definitely. Under the guidance of Ehrlich's lateral chain hypothesis which, broadly speaking, assumes that any cell that takes up or binds antigen can produce the corresponding antibody or antibodies, certain experiments have been made for the purpose of determining whether the tissues at the site of the introduction of antigen respond by a local production of antibody. The tissues in question are the conjunctiva and those of the anterior chamber of the eye; the tissues of the pleura and peritoneum; and the subcutaneous tissues.

THE CONJUNCTIVA AND THE ANTERIOR CHAMBER.

Römer[1] reached the conclusion that on immunization of rabbits with abrin by way of the conjunctiva, the conjunctiva itself furnishes part of the resulting antitoxin at the same time as that abrin which is absorbed into the general circulation calls forth antitoxin

* Received for publication June 10, 1911.

[1] "Experimentelle Untersuchungen über Abrin (Jequiritol)-Immunität als Grundlagen einer rationellen Jequirity-Therapie," *Arch. f. Opthalmol.*, 1901, 52, p. 72.

production in the spleen and marrow. It may be pointed out, however, that at the time when Römer made his tests which showed that the spleen, the marrow, and the conjunctival tissue (of the eye exposed to abrin) contained antitoxin the blood was also antitoxic. One of the rabbits had been under treatment for six weeks, the other for three, and during this time the conjunctiva in question was repeatedly subjected to the action of increasing quantities of abrin. It consequently is not excluded that the antitoxic action of the conjunctival tissue may not have resulted from the passage of antitoxin from the blood and lymph into the conjunctiva. While it is true that the conjunctiva of the untreated eye was devoid of antitoxic power, the repeated attacks of inflammation in the eye exposed to abrin would have rendered much more easy the passage of antitoxin into this conjunctiva. In active as well as passive immunity of rabbits to sheep's blood, Miyashita[1] finds that the lytic amboceptor passes into the normal corneal tissue. This is not surprising in view of the apparently constant presence of antibodies in the lymph when they occur in the blood, the concentration being not far below that of the blood.[2] Here may be mentioned also the observation of Flexner and Clark[3] to the effect that in experimental poliomyelitis of monkeys the specific immune substances, elaborated outside of the nervous tissues, in the earlier stages of the attack escape into the cerebrospinal fluid; later, however, as the vessels return to normal, this passage ceases. Hence the apparent production of antitoxin by the treated conjunctiva as well as its immunity to abrin in Römer's experiments may be explained as a result of the general immunity by virtue of which antitoxin in the blood and lymph was deposited in the conjunctival tissue.

V. Dungern[4] found that so long as the iris was not injured in any way no specific precipitin would appear in the aqueous humor in rabbits immunized with maja-plasma by subcutaneous injection.

[1] "Die Immunitätsverhältnisse der Hornhaut," *Ztschr. f. Immunitätsf.*, 1911, 9, p. 541.

[2] Becht and Greer, "A Study of the Concentration of Antibodies in the Blood of Normal and Immune Animals," *Jour. Infect. Dis.*, 1910, 7, p. 127; Hektoen and Carlson, "On the Distribution of Antibodies and Their Formation by the Blood," *ibid.*, p. 319.

[3] "Experimental Poliomyelitis in Monkeys," *Jour. Am. Med. Assn.*, 1911, 56, p. 585.

[4] *Die Antikörper*, Jena, 1902.

In a number of experiments he first withdrew the contents of the anterior chamber and filled it again with crab-plasma (*Maja-squinado*) diluted four times with water; in some instances the experiment failed for various reasons, notably infection, and in two such cases precipitin appeared in the blood and not in the fluid in the chamber; in two other instances no precipitin was demonstrated either in the blood or the humor; but in one animal the result seemed to indicate that precipitin was formed by the cells about the anterior chamber. In this case a few drops were injected into the chamber of the right eye and eight days later the aqueous humor of this eye gave precipitate while the serum of the blood and the aqueous humor of the left eye gave none at all. The same result was obtained on the following day, and on the day after that the blood serum also caused a precipitate; for a long time thereafter precipitin was present in the humor of the right eye and in the blood. Here it does seem as if in this case precipitin was formed by the cells about the anterior chamber, but v. Dungern's conclusion from this single result that all kinds of cells may form antibodies and his suggestion that in this case the precipitin in the blood also was formed in the anterior chamber are open to doubt.

Proceeding along the same lines I have made some experiments involving the anterior chamber of the eye in dogs. First the aqueous humor was removed by means of syringe and needle, special care being taken not to puncture the iris or any other vascular structure; the anterior chamber of one of the eyes was then filled with a thick and sterile suspension of rat or goat corpuscles, that of the other eye with sterile salt solution. The fluids of the anterior chambers, were removed by puncture from time to time and tested as to their content in specific antibody at the same time as the antibody content of the serum of the blood was determined. So far as the normal aqueous humor is concerned it contains practically no antibody for rat or goat corpuscles; sometimes a trace of opsonin for rat corpuscles is found. In no case did the fluid of the anterior chambers in these experiments give any evidence of the presence of antibody until after newly formed antibodies appeared in the blood, and the amount of antibody in the humor of the injected eye was always much less than that in the blood serum.

In some cases the fluid in the anterior chamber of the control eye also contained antibody but usually not until after the second puncture. In all cases the amounts of antibody in the anterior chambers were greater the more marked the inflammatory reaction.

The general results here outlined are illustrated in the following tables:

TABLE 1.

SHOWING THE CONCENTRATION OF SPECIFIC AGGLUTININ AND OPSONIN IN THE FLUIDS OF THE ANTERIOR CHAMBERS, AND OF THE BLOOD SERUM OF DOGS INJECTED WITH RAT CORPUSCLES IN THE ANTERIOR CHAMBER OF THE RIGHT EYE.

Days after Injection	Experiment 1			Experiment 2			Experiment 3		
	Right Eye	Left Eye	Blood Serum	Right Eye	Left Eye	Blood Serum	Right Eye	Left Eye	Blood Serum
0	0	0	24	0	0	48	0	0	48
2	..	..	24	..	..	96	..	..	..
3	0	0	48	0	0	96	..	..	..
4	0	0	196	0	0	96	0	0	192
5	..	..	..	12	6	96	..	..	..
6	6	0	96	..	..	..	..	..	..
7	..	..	..	12	6	384	..	..	..
8	..	..	..	6	0	384	..	..	..

The figures give the highest dilution in which the fluids in question gave distinct agglutination and opsonification; 0 means no effect in a dilution of 1 to 6.

TABLE 2.

SHOWING THE CONCENTRATION OF SPECIFIC LYSIN IN THE FLUIDS OF THE ANTERIOR CHAMBERS AND OF THE BLOOD SERUM OF DOGS INJECTED WITH GOAT CORPUSCLES IN THE ANTERIOR CHAMBER OF THE RIGHT EYE.

Days after Injection	Experiment 1			Experiment 2			Experiment 3		
	Right Eye	Left Eye	Blood Serum	Right Eye	Left Eye	Blood Serum	Right Eye	Left Eye	Blood Serum
0	0	0	96	0	0	96	0	0	96
2	..	...	...	...	...	...	6	0	192
3	...	...	...	...	...	...	6	6	192
4	192	0	1,536	192	0	384	6	6	384
5	...	...	...	...	...	...	24	12	384
6	...	...	...	384	192	1,536	...	...	...
7	...	...	...	...	...	...	...	...	...
8	...	192	12,288	...	...	...	...	...	...

The figures give the highest dilution in which the fluids in question gave distinct lysis; 0 means no effect in dilution of 1 to 6. The fluids were heated to 58° C. for 30 minutes before tested and fresh guinea-pig serum was used as complement (see p. 107 for quantities).

THE PLEURA AND THE PERITONEUM.

In order to determine whether in rabbits the tissues of the pleura and the peritoneum have the power to produce lysin for typhoid bacilli Wassermann and Citron[1] determined the amount

[1] "Ueber die Bildungsstätten der Typhusimmunkörper. Ein Beitrag zur Frage der lokalen Immunität der Gewebe," *Ztschr. d. Hyg. u. Infektionskr.*, 1905, 50, p. 331.

of lysin in the blood serum and serum of peritoneal and pleural exudates after single injections of bacilli intravenously, intraperitoneally, and intrapleurally. The exudates were produced by means of suspensions of aleuronat. The results obtained are interpreted as indicating the possibility of formation of antibodies by the cells of the peritoneum and the pleura because in some cases the serum of the exudate in animals given pleural and peritoneal injections produced lysis of typhoid bacilli in higher dilutions than the blood serum of those animals. This seemed true especially in the case of the pleura.

I have made similar experiments on dogs, using rat and goat corpuscles as antigens. To obtain exudate, suspension of aleuronat was injected into the pleural cavity, the resulting fluid being withdrawn 10 to 14 hours later and again in most instances at the end of the next 24 hours (Tables 3 and 4). The clear serum obtained after centrifugation of the exudate was used in the tests. In all cases special care was taken to secure exudate free from blood. In the case of the dogs injected with rat corpuscles as well as in the case of those injected with goat blood the final test was made at the same time so that in each case all the samples of serum were tested with the same rat or goat corpuscles as the case might be. The sera were first heated to 50° C. for 30 minutes. The antigoat sera were reactivated with the same guinea-pig serum in the determination of their lytic powers, 0.0125 c.c. of guinea-pig serum being used in each test, the total quantity of each mixture being 0.6 c.c., of which 0.2 c.c. was five per cent suspension of carefully washed goat corpuscles; the remainder of the mixture was $M/8$ NaCl solution, the necessary quantity of immune serum, and 0.0125 c.c. fresh guinea-pig serum. The mixture for testing the agglutinating and opsonic powers of the antirat sera contained simply 0.2 c.c. five per cent suspension of washed rat corpuscles, the requisite amounts of immune serum, and sufficient salt solution so that each mixture equaled 0.6 c.c. The preliminary tests made immediately after the sera were obtained gave the same results as those given in the tables.

In Table 5 are given results obtained with the sera of dogs injected with goat blood and killed 3, 6, 9, and 12 days after the

TABLE 3.

SHOWING THE AMOUNT OF ANTIBODY (AGGLUTININ) IN THE BLOOD AND IN PLEURAL EXUDATE AFTER THE INJECTION OF RAT CORPUSCLES (1 C.C. 10 PER CENT SUSPENSION PER KILO OF WEIGHT OF DOG) (*a*) INTRAVENOUSLY AND (*b*) INTRAPLEURALLY.

Days after Injection	Intravenous Injection of Antigen				Intrapleural Injection of Antigen			
	Dog I		Dog II		Dog I		Dog II	
	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate
0	96	....	48	....	96	....	48	....
2	24	....	48	....	96	....	48	....
4	96	....	96	....	192	....	48	....
5	768	384	768	....	384	....	192	384
6	1,536	....	1,536	....	768	....	768	....
7	3,072	1,536	3,072	1,536	....	....	1,536	1,536
8	6,144	3,072	6,144	....	3,072	....	3,072	....
9	6,144	3,072	3,072	3,072	3,072	3,072	3,072	3,072
11	6,144	3,072	6,144	6,144	3,072	3,072	1,536	1,536
13	6,144	3,072	6,144	6,144	6,144	3,072	1,536	1,536
15	3,072	....	6,144	....	3,072	1,536	1,536	....
18	3,072	....	6,144	....	3,072	....	3,072	....
20	3,072	3,072	3,072	....	3,072	1,536	3,072	1,536
24	3,072	....	3,072	....	3,072	....	3,072	....
28	Killed	....	3,072	....	3,072	....	3,072	....
33	....	....	3,072	....	Killed	....	1,536	....
38	....	....	3,072	....	....	....	768	....
39	....	....	3,072	1,536	....	....	768	768
45	....	....	3,072	....	....	....	768	....
52	....	....	3,072	....	....	....	192	....
59	....	....	1,536	....	....	....	192	....
66	....	....	768	....	....	....	192	....
73	....	....	768	....	....	....	192	....
80	....	....	768	....	....	....	192	....

The figures give the highest dilution in which the sera were active. Determinations of opsonin at various times gave results in exact harmony with those in the table.

TABLE 4.

SHOWING THE AMOUNT OF ANTIBODY (LYSIN) IN THE BLOOD AND IN THE PLEURAL EXUDATE AFTER THE INJECTION OF GOAT CORPUSCLES (1 C.C. 10 PER CENT SUSPENSION PER KILO OF WEIGHT OF DOG) (*a*) INTRAVENOUSLY AND (*b*) INTRAPLEURALLY.

Days after Injection	Intravenous Injection of Antigen				Intrapleural Injection of Antigen			
	Dog I		Dog II		Dog III		Dog IV	
	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate
0	24	....	24	....	24	....	24	....
2	24	....	24	....	48	....	24	....
4	1,536	768	1,536	768	768	768	1,536	768
5	24,576	....	6,144	3,072	3,072	768	12,288	6,144
7	49,152	....	49,152	....	6,144	....	24,576	....
9	49,152	49,152	24,576	12,288	12,288	3,072	24,576	12,288
10	49,152	49,152	24,576	24,576	6,144	3,072	49,152	12,288
12	49,152	....	24,576	....	6,144	....	24,576	....
14	24,576	24,576	12,288	12,288	3,072	1,536	12,288	....
15	49,152	24,576	12,288	6,144	6,144	1,536	....	....
17	24,576	....	6,144	....	6,144	....	....	....
19	12,288	6,144	6,144	6,144	6,144	1,536	....	....
21	12,288	....	6,144	....	3,072	....	....	....
24	12,288	....	6,144	....	3,072	....	....	....
28	6,144	....	6,144	....	1,536	....	....	....

The figures give the highest dilution in which the sera were active.

injection, aleuronat suspension being injected into the pleural and peritoneal cavities about four hours before the animals were killed. In this case peritoneal exudate was also tested. It should be noted that in the experiments represented in this table the exudates were obtained much earlier than in those represented in Tables 3 and 4, and furthermore, that in the animals thus killed the effects of the injection of aleuronat suspension on the leukocytic content of the blood and hence on the antibody-forming functions of the hematopoietic organs necessarily would be much less than in the cases of the animals that lived on after receiving the injections of aleuronat.

TABLE 5.

Showing Concentration of Antibodies in Blood Serum and Exudate Serum after Intrapleural and Intravenous Injections of Goat Blood in Dogs (1 c.c. 10 per cent Suspension of Goat Blood per Kilo of Dog).

I. *Intrapleural Injection.*

Days after Injection	Blood Serum			Pleural Exudate			Peritoneal Exudate		
	Lysin	Agglutinin	Opsonin	Lysin	Agglutinin	Opsonin	Lysin	Agglutinin	Opsonin
3	192	12	12	96	12	6	...	...	...
6	768	48	96	384	24	48	384	24	48
9	6,144	192	192	768	24	24	...	...	...
12	384	12	48	192	12	24	96	12	12

II. *Intravenous Injection.*

Days after Injection	Lysin	Agglutinin	Opsonin	Lysin	Agglutinin	Opsonin	Lysin	Agglutinin	Opsonin
3	384	24	24	...	...	...	...	...	...
6	24,576	192	384	6,144	96	96	6,144	96	96
9	24,576	192	192	6,144	96	96	...	...	...
12	6,144	96	192	...	...	...	...	...	...

In this experiment the dogs were killed at the end of 3, 6, 9, and 12 days after the injections; the exudates were obtained about four hours after the injection of aleuronat. The opsonin determinations were made with leukocytes from pleural exudates in dogs, and carefully washed in order to free them from serum.

The results of these experiments indicate that in most cases the intrapleural injections of the antigen give less concentration of antibody in the serum of the blood than the intravenous injections. Furthermore, that in no case after intrapleural injections of antigen did the content of antibody in the serum of the pleural exudate exceed that in the serum of the blood; often it is a little less. The relation between the antibody content of the exudate and that of the blood seems to be almost the same whichever way the antigen was introduced.

SUBCUTANEOUS TISSUES.

Wassermann and Citron injected dead typhoid bacilli into the ears of rabbits and ligated the ear for two hours; they determined the lytic power of the blood serum on the tenth day, and then amputated the ear. In one rabbit of several thus treated there was a much greater and more rapid fall after the amputation than in the control animals; and this result they hold points to a local production of antibody by the connective tissue of the ear. Inasmuch as many determinations were not made, so that a comprehensive idea of the course of antibody production in these animals could have been obtained, this result alone cannot well be given any decided significance.

That specific vaccine may cause antibody production in a body that is already infected is referred by Wright[1] and others to induced activity of the tissues at the site of the injection, and that is usually the subcutaneous tissue. This view is voiced very clearly by Russell[2] in his explanation of how typhoid vaccine is beneficial in typhoid fever. He holds that the subcutaneous tissue produces antibodies freely, and that as it is not ordinarily involved in typhoid fever, subcutaneous injections of vaccine in this disease do good by throwing this unused center into action.

Forssman[3] and others have observed that the rate and manner of production of certain antibodies, as shown by the curves obtained with the serum of the blood, seem to depend to some degree on the site of the injection of the antigen. For example, the antitoxin curve obtained after subcutaneous injection of botulismus toxin would differ somewhat (but not radically) from that obtained after intravenous injection of the toxin. Regarding this fact as evidence that distinct cell groups are thrown into action as the injections are made in different places, Forssman suggests that each distinct tissue gives a curve of its own. It is possible, however, that the differences noted, is so far as they are not due to individual variations in the power to produce antibodies, depend simply on differences

[1] *Studies on Immunization*, 1909.

[2] "The Prevention and Treatment of Typhoid Fever with Antityphoid Vaccine," *Bost. Med. and Surg. Jour.*, 1911, 164, p. 1.

[3] "Studien über Antitoxinbildung bei aktiven Immunisierung gegen Botulismus," *Centralbl. f. Bakt.*, I Orig., 1905, 38, p. 463.

in the rate and concentration in which the antigen is carried from the places of introduction to the centers where antibodies are produced.

If antibodies are produced locally at the site of injection of antigen into the subcutaneous tissue, then it would be reasonable to expect that subcutaneous injections of antigen in many places would give large production of antibodies. Accordingly two dogs were injected with one c.c. per kilo of body weight of 10 per cent suspensions of foreign corpuscles, using goat corpuscles in one case and rat in the other, in 10 different places. In neither case did the resulting antibody curve exceed, or differ materially from, the curves that are produced by a single injection subcutaneously of the same quantity of goat or rat blood. Manifestly much weight should not be placed on this result unless corroborated by the results of other experiments of a like nature but with different dosage of antigen.

Wright[1] suggests that massage after the injection of a vaccine into a limb might give such increase of antibodies in the blood that all doubt as to their local production would be removed. Accordingly rat or goat corpuscles were injected into the muscles of the foreleg in dogs, and when the production of antibodies was well under way the tissues injected were subjected to skilled massage (Dr. Carlström) for 20 minutes, but in no case did the antibody content of the blood carefully tested at hourly or half-hourly intervals after the massage, show any such decided rise as to indicate any production of antibodies at the site of injection of the corpuscles.

Again, if the tissues at the site of the introduction of antigen take an effective part in the production of specific antibodies, then there should be found a distinct difference in the amount of antibody in the blood of animals in which these tissues are removed in the early part of the course of antibody formation as compared with the amount in other animals in which the same tissues are not removed, all other conditions remaining the same as nearly as possible. In order to test this hypothesis experiments were made on dogs injected with goat blood, which in this animal

[1] *Studies on Immunization*, 1909.

induces, as already indicated, a comparatively liberal production of specific antibodies.

The experiments were made as follows: The same quantity of goat blood in proportion to weight—one c.c. of a 10 per cent

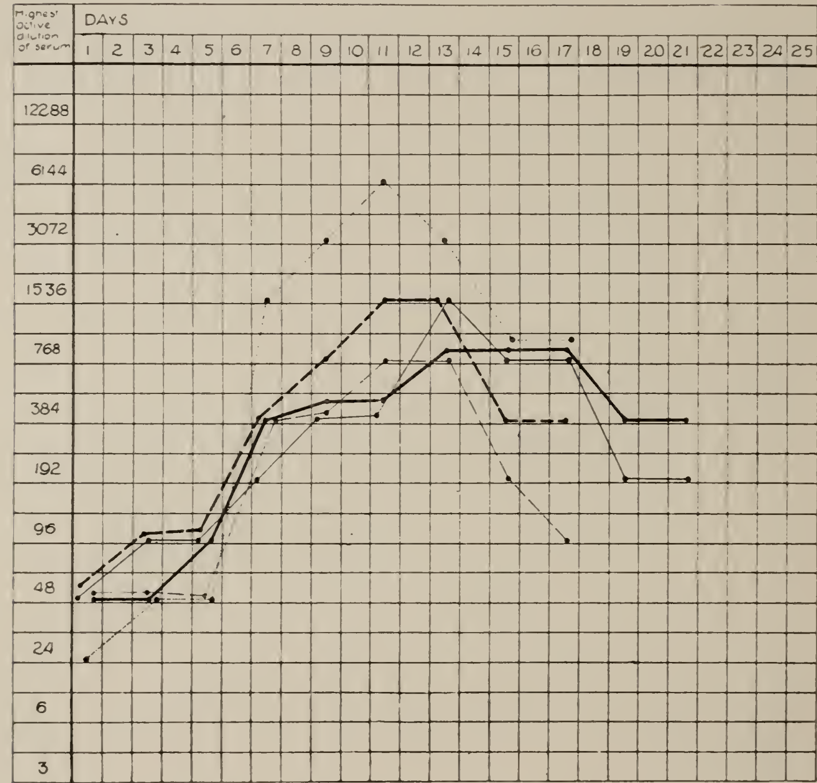

CHART 1.—Effect of amputation of foreleg injected with antigen on the production of antibodies.
Heavy lines—Injection of antigen in right foreleg; amputation of right foreleg two days later.
Fine lines—Injection of antigen in right foreleg; amputation of left foreleg two days later.
Dotted lines—Injection of antigen in right foreleg; no amputation.

suspension per kilo of dog—was injected subcutaneously in the lower part of the right foreleg of each of five dogs; two days later the right foreleg was amputated in two of the dogs, and the left in two, while in the case of the fifth dog nothing was done to disturb or modify the formation of antibodies. The subsequent events in the dogs subjected to amputation ran practically the same

course; in all there developed some suppuration in the stumps, but in the two represented on Chart 1 by the broken lines this was quite insignificant, and in no case did the general condition appear to be affected. The results so far as the specific lysin in the blood of these dogs is concerned are shown in the chart which shows that there was no striking or definite difference in the amount of lysin in the blood of those dogs whose right forelegs were amputated, as compared with that in the blood of those that lost their left forelegs; in all four the amount is distinctly less than in the fifth dog which was left undisturbed. These results do not indicate that the tissues at the site of the injection of the antigen took any measurable part in the production of antibody.

SUMMARY.

The injection of rat or goat corpuscles in the anterior chamber of the eye of dogs is followed by the appearance of specific antibodies in the blood and usually in the aqueous humor. The concentration of the antibodies is greater in the humor of the injected eye than of the uninjected, but in every case it is much less than in the blood. The antibodies do not appear earlier in the aqueous humor than in the blood.

The injection of rat or goat corpuscles in the pleural cavity in dogs is followed by the appearance of specific antibodies in the blood and in pleural exudates evoked by means of aleuronat. The concentration in the blood is probably a little lower than after intravenous injection of the same amount of antigen. The concentration in the pleural exudates is not higher than in the blood, often it is less. There is no difference in the relation between the antibody content of the blood and of the pleural exudate in dogs receiving the antigen in the pleural cavity in question and in dogs receiving the antigen intravenously.

Massage of the tissues at the site of injection of antigen does not seem to increase the antibody content of the blood in dogs.

In dogs injected subcutaneously over the foreleg with rat or goat corpuscles amputation of the injected leg in the early phases of antibody formation does not result in less antibody in the blood

than in other dogs in which the same tissues are not removed, the other conditions being equal.

The results obtained from the experiments recorded in this article consequently do not point to any local production of specific antibodies in dogs injected with goat or rat corpuscles, at least not so far as concerns the tissues about the anterior chamber of the eye, the tissues of the pleura, and the subcutaneous tissues.

THE RESISTANCE OF TUBERCLE BACILLI TO DRY HEAT.*

CHARLES KRUMWIEDE, JR.
(From the Research Laboratory, Department of Health, New York.)

Although as a general rule all bacteria are more resistant to heat when dry, the degree of resistance of the tubercle bacillus to dry heat as given seems very great. Thus, Schill and Fischer[1] used sputum dried 98 or 142 days and failed to infect pigs after one hour's heating. A sputum dried only five days infected all the pigs after one-half hour's heating. After one hour's heating only one of three pigs became tuberculous.

Grancher and Ledoux-Lebard[2] conclude from their experiments, that heating to 100° C. for two or three hours reduces the virulence of cultures but does not destroy it. A comparison of these two reports shows little agreement. A repetition of the work therefore seemed advisable.

The material for the test was from eight different recently isolated human cultures on glycerine egg. The cultures were about three weeks old. The bacilli were removed, mixed, and ground up in an agate mortar. They were then dried in a thin layer in the incubator for 24 hours, the object being to approximate practical dry air sterilization with air-dried contaminated material, and again ground up in the mortar to a fine dust-like condition. This grinding was sufficient to break up clumps of any size, but not sufficient to break up the bacilli. The material was placed in narrow test tubes which were then drawn out to capillary size and bent at the upper end in an acute angle. Approximately 0.025 gm. of culture was added to each tube. The culture was therefore in the bulb-like extremity of the tube, the capillary part giving free exit of air, and the upper end—the diameter of the original test tube, but bent down—was plugged with absorbent

* Received for publication May 3, 1911.

[1] *Arb. a. d. k. Gsndhamte*, 1884, 2, p. 131.

[2] *Arch. de méd. expér. et d'anat. path.*, 1892, 4, p. 1.

cotton. A large pail of water was heated to boiling and the bulbs suspended in the water by hooking the bent tube over the side of the pail. The bulbs were protected from the side of the pail by a thick layer of cotton. The openings of the tubes being bent downward were protected from the steam which rose upward due to the draft in the hood. A thermometer suspended in the water registered 100° C. The same thermometer when put into a tube of the same size as used for the tests registered about 0.2° less. As this tube was open, one is safe in assuming that in the tubes with a capillary opening only, the temperature was practically 100° C.

After heating, the bacilli were suspended in a salt solution and injected intramuscularly in guinea-pigs. The results are given in the accompanying table.

Time	Pig A	Pig B	Notes
0: control	++++	++++	Marked generalized tuberculosis
5 minutes	++++	+++	Approximately same as control
10 minutes	++++	++++	Approximately same as control
20 minutes	++	++	Lesions decidedly less than in control
45 minutes	....	Died acutely	No local caseation at site of injection
1 hour, 15 minutes	....	....	No local caseation at site of injection

Evidently there was already some diminution in the number of bacilli at 20 minutes. Complete destruction however took place in between 30 and 45 minutes.

Air-dried tubercle bacilli are therefore many times more resistant to dry heat than are bacilli heated in fluids or steam.

THE EFFECT ON THE PROTOPLASM OF NITELLA OF VARIOUS CHEMICAL SUBSTANCES AND OF MICRO-ORGANISMS INTRODUCED INTO THE CAVITY OF THE LIVING CELL.*

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Many experiments have been conducted to test the effect on unicellular plants and animals of various substances introduced into the fluid surrounding the cells. In the experiments described in this paper, the test substances were introduced by means of very fine glass capillary pipettes directly into the vacuole or protoplasm of the living cell. By the use of this method the observer may note the effect of the substances when brought into immediate contact with the protoplasm or when introduced into the vacuole, where the only barrier to diffusion into the protoplasm is the protoplasmic layer immediately lining the vacuole. Further, one may test the local effect on the part of the cell into which the substance is injected. The results differ materially from those obtained by applying the chemical outside of the wall, especially as regards the local effects observed.

The technic employed has been described in a previous paper.[1] In this method the cell wall is pierced with pipettes which may be made so small that the lumen scarcely exceeds one micromillimeter in diameter. Such a pipette may be introduced into the relatively large cells of Nitella with little or no injury to the cell. The expansion of mercury behind the substance to be injected furnishes the force necessary for overcoming the cell pressure, and a device for applying heat or cold to this mercury serves to approximately regulate the size of the dose.

Nitella was chosen as a test cell because of its relatively large size, the transparency of its wall, especially in the rhizoids and the portion of the main filament next to the rhizoids, and because of the rapid movement of its protoplasm, which gives the observer a

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[1] *Jour. Infect. Dis.*, 1911, 8, p. 348.

criterion of the effect of the substances introduced. The size of the cells varied from 0.5 mm. in diameter and 50 mm. in length to those 0.2 mm. or less in diameter and of proportional length. The term "cell" is here used for the cornocyte consisting of many nuclei in a common cytoplasm.

The size of the dose was only approximately measured. It varied from a globule of liquid two or three micromillimeters in diameter to quantities approximating $\frac{1}{10}$ of the total volume of the cell. In the table below the expression "small" refers to doses a few micromillimeters in diameter; and the "large" doses as a rule exceed $\frac{1}{100}$ of the volume of the cell. These are only rough estimates but may give some idea of the amounts injected.

It was found that fairly large doses of water, physiological salt solution, or broth had little or no effect on the cells, at most causing only a temporary slowing of the movement of the protoplasm. Very large doses of any substance, suddenly introduced, sometimes caused the death of the cell, possibly as much through mechanical insult as through any purely chemical action. As a rule the action of the chemical injected was at first only local, and in most cases the effect of smaller doses was restricted to local injury.

In many cases there occurred the death or paralysis of only a small portion of protoplasm in immediate contact with the substance injected. There was often in these cases a temporary heaping of protoplasm about the point of injection with a subsequent carrying away of the injured protoplasm and a return of the cell to normal flow. When the toxic effect was a little stronger, enough destruction of protoplasm was effected to form a plug of dead protoplasm often sufficiently large and compact to divide the cell into two parts, each part living and possessing its individual mass of rotating protoplasm. This plug sometimes persisted for hours, and in one case finally disintegrated, leaving the cell normal, except for a mass of dead protoplasm at the point of inoculation, and some fragments of dead protoplasm floating in the protoplasmic current. Finally, with larger doses or stronger poisons, not only local destruction of protoplasm occurred, but cessation of movement in the entire cell soon followed. Unless overwhelming doses

were employed, even so powerful a cell poison as bichloride of mercury did not instantly kill the cell, but a local necrosis was followed some minutes later by the stoppage of the protoplasm of the entire cell. The local death of the protoplasm prevents the poison from being quickly carried by the protoplasmic stream to other parts of the cell. The killed protoplasm in the immediate neighborhood of the point of injection usually became less transparent and took on a light brownish color. It sometimes became more vacuolated.

In these experiments it was, of course, necessary to prevent the complication of results by the accidental ejection of the chemical tested outside as well as inside of the cell. To guard against this possibility enough time was usually allowed after the insertion of the pipette to make sure that the flow of the protoplasm was normal before injecting the dose. In the case of strong poisons the tip of the pipette was often washed in water before inserting it into the cell. In several experiments other cells lay near enough to the one tested to serve as controls as regards the presence of the chemical outside of the cell.

Below is given a table in which the chemical substance, the approximate dose, the number of tests, and the effect on the cell are given. Substances are arranged in the table approximately in the order of their toxicity for the cell. The permanent stoppage of protoplasmic movement throughout the entire cell was taken as an indication of the death of the cell. Unless otherwise stated, solutions were made with distilled water.

In reviewing the results of these injections it is noteworthy that strychnine, alcohol, arsenic, and quinine had comparatively slight effect on the cell protoplasm. Saturated solution of copper sulphate in small doses may be introduced into the vacuole of the cell with little effect. Since the protoplasmic layer immediately lining the vacuole opposes some resistance to substances entering from the vacuole, the effect of a substance inoculated directly into a vacuole may be compared with that of a high dilution outside of the cell. Chloroform and ether exerted a powerful local effect, but not so great a general effect as bichloride of mercury or osmic acid. It is to be noted that the amount of chloroform and ether injected was exceedingly small. A tiny globule was forced out

TABLE 1.

Substance Injected	Concentration	Size of Dose	Number of Tests	Effect on Cell of Nitella
Bichloride of mercury	Saturated solution	Medium	1 2	Local death of protoplasm followed by death of entire cell in about 10 minutes.
		Small	2	In one case plug of dead protoplasm with living protoplasm on either side. In other case death of entire cell.
Bichloride of mercury	Crystals ground in olive oil	Large	2	In one case early death of cell. In other case cell survived about 3 hrs. Apparently a slow dissolving out of mercury from the oil.
Osmic acid	Saturated solution	Medium	2	Instant local destruction of protoplasm followed in a few minutes by death of the entire cell.
Potassium hydroxide	50 per cent	Small		Action like that of saturated solution of bichloride of mercury.
Copper sulphate	Saturated solution	Large and small	11	Very small doses tolerated. Larger doses followed by local necrosis of protoplasm and later by death of entire cell.
Formaldehyde	About 4 per cent	Small	1	Local death of protoplasm with formation of plug of protoplasm dividing cell into two living parts.
Formaldehyde	40 per cent	Small	1	Local death of protoplasm followed by death of entire cell in about 10 minutes.
Chloroform	Undiluted	Very small	2	Plugs of dead protoplasm formed with living cell segments on either side. In one case the plug after several hours disintegrated and segments were united.
Chloroform	1 per cent	Varying	4	Very small doses followed by local necrosis only. Large doses, local death followed in a few minutes by the death of entire cell.
Ether	Undiluted	Small	2	Action much like chloroform but less poisonous.
		Large	1	Local death soon followed by death of whole cell.
Alcohol	Absolute	Varying	7	Only large doses caused much local death of protoplasm. Small doses effected no apparent harm.
Alcohol	95 per cent	Large	5	Little toxic effect.
Arsenic trioxide	Saturated solution	Small and large	3	Little effect of small doses. Larger cause some local destruction of protoplasm.
Fowler's solution	....	Varying	12	Little if any effect of large doses.
Sulphate of quinine	Saturated solution	Varying	3	Local death follows large doses. Toxic effect small.
Sulphate of strychnine	1/500 to 1/10,000	Varying	10	No effect.
Sulphate of strychnine	Saturated solution	Large	4	Apparently no effect.
Sodium chloride	Saturated solution	Medium	..	Slight local destruction of protoplasm, no stoppage of flow.
Sodium chloride	0.85 per cent	Large	.	No effect.
Mercury metallic	....	Globules minute to about ½ diameter of cell	Many	No effect unless large enough globules to cause mechanical injury.
Methylene blue	Nearly saturated	Very large	Many	Very little or no poisonous action.
Water	....	Varying	Many	No effect.

into contact with the protoplasm, and, after a minute or so of contact, was withdrawn into the pipette again; so the cell received only the minute quantity that could diffuse from the globule in that very short time. Yet this amount was sufficient to cause the death of a considerable mass of protoplasm. Bichloride of mercury may be injected in small enough dose to cause only local death of the cell.

In some experiments a dose of methylene blue was injected immediately after some protoplasm-destroying agent. It was found that there was no immediate deep staining of the protoplasm, but at most only a stain of the protoplasm that was in immediate contact with the fixative. From this it seems likely that the mere stoppage of protoplasm does not immediately result in its death, or at most does not bring it at once to the stage when it readily takes up dyes. No attempt was made to ascertain if a cell may be rendered tolerant to a poison by small repeated doses. It seemed that this might be more conveniently and accurately done by applying solutions from the outside, though it might be worthy of an experiment to compare the action of substances thus applied with the action when brought directly into the cell cavity.

Some of this work was done while the technic was in the process of evolution and it is recognized that the work lacks definiteness in respect to measurement of the size of the dose, a thing which could be done much more accurately at the present stage of the development of the technic. But it has seemed to the writer that a new province of experimentation has been opened by this method and that this paper may be worth while at least as an introduction to this field of research.

II. THE INFECTION OF LIVING CELLS BY MEANS OF MICROORGANISMS INTRODUCED WITHIN THE CELL CAVITY

The most of this work[1] has been done on plant cells, though some experiments were carried out with the larva of the gnat, *Chironymus*. Aquatic plant cells of large size were for the most part employed, Nitella and Vaucheria of the green plants, and the fish mould genera, Saprolegnia, Achlya, and Dictyuchus of the fungi. These cells were chosen because of their relatively large size, their transparency, and the active circulation or rotation of their protoplasm which gives the observer some criterion of the condition of the cell.

The microorganisms inoculated were vegetating bacteria, spores of bacteria, yeast plants, and spores of fungi. The dose varied from several organisms to hundreds. As a rule no immediate injury to the cell inoculated could be noted following the injection of the dose, even when relatively large quantities of well grown broth cultures were used.

During inoculation the cells were kept in shallow hanging drops in water, and after inoculation were transferred to watch glasses, or kept in moist chambers in hanging drops. Uninoculated cells kept in the same condition served as controls.

The prevention of injury to the cells pierced and the ease with which the wall may be penetrated depend much on the size and character of the point of the pipette. With very fine pipettes, sufficiently large for injecting small bacteria, the cell wall of Nitella or of the fish moulds may be pierced with no disturbance of the flow of the protoplasm. Larger bacteria, yeasts, and the spores of fungi require pipettes of larger openings. Such pipettes sometimes cause loss of protoplasm with a temporary checking of the flow on withdrawal of the pipette after inoculation, but unless the opening made is very large or the wall torn in piercing, it is quickly

[1] Later work with this technic has emphasized the importance of using the abruptly tapering pipette points described in a previous paper. The extension of a part of the glass tube below the loop as described in Fig. 1, *n* (see *loc. cit.*), has been found to be unnecessary. Further, it will perhaps be more convenient for most workers to hold the pipette in the right hand and the forceps in the left in drawing the fine point of the pipette.

closed by protoplasm and no lasting injury to the wall results. In the larger Nitella cells some difficulty may be met with in piercing the tough walls with the larger pipettes. If the end of the pipette is broken off slantwise instead of square across, it enters more easily; and if the tip be placed against the wall, some pressure applied, and the mechanical stage moved back and forth, a boring action is obtained which aids much in penetrating the cell.

When the larger pipette is withdrawn there tends to be an outrush of cell contents owing to the high cell pressure. This outflow may carry with it the material inoculated, or, if the opening is large, may result in too great a loss of contents. In the fish moulds the pipette may be left in the cell until a mass of protoplasm has formed over the tip. The pipette may then be withdrawn without a particle of loss of contents. In case of cells with rapidly rotating contents, as Nitella, the dose injected is soon swept so far away from the point of inoculation that there is little danger of its loss on the withdrawal of the pipette. Considerable loss of contents may be borne by the cells of this plant without apparent injury.

The outflow of contents when a small opening is made in the cell continues until the opening is plugged. This prevents the introduction of bacteria from the surrounding medium or from any source except from out of the pipette. In no case in the experiments described here has an accidental contamination been noted. As controls many cells were pierced but not inoculated. These remained in good condition, in some cases for eight or more days after being pierced.

When pipettes of large opening have to be used, there is sometimes danger of the retreat of a part of the dose injected into the pipette when the pressure is stopped. This may be avoided by regulating the degree of contraction of the mercury in the pressure apparatus. When the tip of the pipette is in a mass of protoplasm, the viscid protoplasm will often act as a valve, closing the mouth of the pipette when pressure is diminished.

In the work described here something over fifty inoculations were made in which bacteria were seen to multiply in the still living cell. No cell was classed as living unless movement of protoplasm could be distinctly made out.

The list of cells inoculated and organisms injected are as follows:

Cell	Organism	Number of Tests Made
Nitella	B. prodigiosus	8
Vaucheria	B. prodigiosus	1
Nitella	B. typhosus	5
Nitella	B. subtilis, rods	3
Nitella	B. subtilis, spores	1
Nitella	Sporotrichum	1
Nitella	Yeast	4
Saprolegnia	Water bacteria	11
Saprolegnia	B. typhosus	2
Achlya	B. typhosus	7
Achlya	B. prodigiosus	1
Dictyuchus	Water bacteria	3
Dictyuchus	B. coli communis	2

It was not always easy to identify the different genera of the fish mould before fruit was formed; but as all behaved nearly alike, an exact botanical classification may not be so important. The Nitella cells chosen were for the most part the more transparent cells found just above the insertion of the rhizoids.

The results of the inoculation of bacteria into both the fungi and the Nitella are so similar that it is hardly necessary to describe each combination in detail. In nearly every case the bacteria grew luxuriantly in the cells, apparently finding there a good medium for growth. Mobile forms generally showed a high degree of motility in the infected cell. In a few cases bacteria apparently living failed to grow, but this might occasionally happen in the transfer of a small number of individuals to a very different medium in a culture tube. As might be expected, the larger the dose the more surely and quickly the cell became infected. Infection followed the introduction of both vegetative forms and spores. In a Nitella filament inoculated with the spores of *B. subtilis* germination of spores was delayed nearly two days at a room temperature of about 26° C. as compared with a control inoculation in broth.

As a rule there was no evidence of a harmful action of the protoplasm of either the fungi or of the green plants on bacteria. Both *B. typhosus* and *B. prodigiosus* swam about freely in the rapidly rotating protoplasm of Nitella. Their movements with respect

to the flowing protoplasm showed that many of them were actually in the protoplasm and not in the vacuole of the cell. They would start upstream in the current of protoplasm, succeed in stemming it for a time, finally yielding and being carried down or forced to take some different direction. Their movements seemed to be embarrassed only by the rapidity of the current and the viscidity of the medium, not by any bactericidal power inherent in it. The bacteria apparently meet with no resistance in passing from the cell vacuole into the protoplasmic layer. No case was observed of bacteria passing from one living cell to another, although in many experiments living uninoculated cells immediately adjoined infected cells in the same chain.

In order to further test the effect of protoplasm on bacteria, two Saprolegnia filaments were inoculated with typhoid bacilli, kept at room temperature until the typhoid had well started, then transferred to a refrigerator temperature of about 12° C. At this temperature typhoid bacilli multiply very slowly, if at all, while the fish moulds grow well; so that any tendency on the part of the Saprolegnia protoplasm to destroy the bacteria would be favored. It was observed that the typhoid bacilli multiplied little, but remained actively motile and healthy in the vacuole of the fungus cell for five or six days. A cell of Nitella was also inoculated with typhoid bacilli, left at room temperature of 21°–22° C. for 20 hours until the bacilli were well started, and then transferred to the refrigerator for 74 hours. On bringing the filament to a temperature of 24°–25° C. the bacilli multiplied freely. The Nitella protoplasm continued to rotate actively at refrigerator temperature. In this case, too, the protoplasm did not destroy the bacilli, even though these were at a temperature relatively more unfavorable to them.

As to the effect of the bacteria on the plant cells, the green plants showed the least resistance to infection, generally dying within 12 to 20 hours at room temperature after the bacteria became numerous in the cells. The fish moulds showed rather more resistance, possibly because of the fact that in their natural environment they are usually surrounded by many bacteria and must have become somewhat accustomed to their products. In

one case, Dictyuchus inoculated with *B. coli communis*, the cell survived 58 hours in the presence of numerous bacteria in the vacuole. The temperature in this experiment ranged from 20°–27° C. It is noteworthy that the plant cells could live many hours with rapidly circulating protoplasm, when bacteria were so numerous in the vacuole of the cells as to form a thick emulsion. Apparently the toxic effect of the bacteria on the protoplasm is relatively slight; and the injury to the cells results largely from parasitism. One must take into consideration the fact that if toxin is produced it would not remain long in contact with the cell protoplasm, since it would readily diffuse out from filaments wholly immersed in water.

As a rule, the protoplasmic layer became thinner and thinner in the infected cells until its motion ceased. Plasmolysis and the death of the cell followed soon after cessation of motion of the protoplasm.

The fish moulds showed some reaction to infection by walling off the more infected portions by partitions in the filament. In other cases the infected cell transformed itself into a sporangium containing bacteria-free spores. In one cell a mass of protoplasm much larger than a spore was segregated off from the bacteria. In another case a bacillus, apparently motile, was included within a spore. Sometimes bacteria-free branches grew out from the protoplasmic layer lining the infected cells. The Nitella and Vaucheria inoculated showed no such reactions to infection.

In a filament of Achlya infected with slowly growing filamentous typhoid bacilli the protoplasm of the infected cell put out tongue-like processes toward the bacilli, suggesting an attempt at phagocytic action. No distinct engulfing of bacteria could be made out, so there may have been no relation between the form of the protoplasmic tongues and the bacteria. From a Nitella cell, the cell still living and containing numerous prodigiosus bacilli, some bacilli were withdrawn by means of the pipette, and sown on agar. No growth occurred. Though a hundred or so of the bacteria were transferred it may be that not enough were taken to give a fair test of viability. The bacteria in this case were non-motile in the cell.

In summary it appears that these plants depend on the barrier afforded by their walls, or, in case of a break in the wall, by protoplasmic membranes, to protect themselves against infection; and their protoplasm has very slight if any antibacterial power. On the other hand their protoplasm opposes a marked resistance to the action of bacterial products, a characteristic perhaps acquired from the frequent exposure of these cells to decaying substances in their

FIG. 1.—A living cell of Nitella containing a large colony of yeast, the darker body in the center, with a mass of protoplasm on either side. The striated appearance at the margins of the cell shows where the protoplasmic stream was most rapid. (Magnification ×160.)

natural habitat. In the presence of a strong cell pressure alone these cells have a protection against introduction of bacteria through some small opening, a protection not shared by plants or animals consisting of masses of cells whose intercellular spaces offer a breeding place for bacteria.

There was but one successful inoculation of a cell with fungus spores, that of Nitella with spores of a Sporotrichum obtained from lesions in the human subject. Small mycelia, at first movable in

the rotating protoplasm, developed on the second day. Later the mycelia increased in size and became fixed in the cell. The cell survived about four days, and during this time no spores formed on the fungus.

Possibly the most remarkable results in the whole series were obtained in Nitella inoculated with yeast cells. Four cells were successfully infected with a pure culture of wild yeast obtained from rancid butter. The yeast cells multiplied rapidly in the living protoplasm and vacuole of the cell at room temperature and more slowly at refrigerator temperature. Finally the cells became so closely packed with yeast cells that the contents of the vacuole resembled a thick emulsion where the cells were so numerous as to be practically in contact with one another. In one cell (see Fig. 1) there was at first apparently a single group of yeast plants in the cell. This grew to form a mass with a diameter nearly as great as that of the Nitella, and with edges somewhat rounded by its rolling movement in the protoplasm. Of the four cells one survived six days, one seven days, one eight days, and one ten days. The greater part of this time the plants were kept at refrigerator temperature. The Nitella cells poorly supplied with chlorophyll, and kept in the dark most of the time, could elaborate little food; and it is remarkable that they could harbor such a mass of foreign organisms for so long a time.

SUMMARY.

1. Bacteria, yeasts, and Sporotrichum find a good medium for growth in the interior of certain living plant cells.

2. There is no evidence of an antibacterial property of the protoplasm of these plants.

3. The protoplasm of these cells shows a marked resistance to the products of bacteria and yeasts, and the death of the cell seems due to parasitism rather than to toxins.

The experiments with animal cells thus far are few. Several successful inoculations of the partially transparent larva of the gnat, *Chironymus*, may be mentioned. These larvae were only about five mm. long, and the difficulty of inoculation was increased by the activity of the animal and the toughness of the skin. Larvae

were infected with *B. prodigiosus*, the bacteria multiplying in the body cavity but not penetrating the intestine during life. These animals showed a remarkable resistance to the action of bacteria. One larva lived 46 hours with *B. prodigiosus* swarming in the body cavity. Controls showed that the mere piercing of the skin is not necessarily followed by infection. Bacteria recovered from the body of an infected animal proved to be the species inoculated.

FURTHER STUDIES OF ANTISTREPTOCOCCUS SERUM.*

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In June, 1909, in the Section of Pharmacology and Therapeutics of the American Medical Association,[1] together with Dr. L. Hektoen, we made a brief preliminary report on a study of various commercial antiserums and vaccines. An immediate resumption of the investigation was prevented by other work, but during the past few months a further study of antistreptococcus serums was undertaken, and the results obtained and now reported are in some respects at variance with those secured in our former work. In the earlier experiments we failed to demonstrate that animals into which antistreptococcus serums were injected possessed any increased resistance to subsequent injections of streptococci. Our efforts to demonstrate streptococco-opsonins in the serums usually met with negative results. At times we were inclined to think that opsonic determinations made by Wright's method pointed toward the presence of specific opsonins in the serums when mixed with fresh serum. However, the opsonic indexes which were obtained were so little above the normal and appeared so inconstant that they were considered as coming within the limits of error in the method employed. The same was true as regards determinations of opsonins in the blood of animals after injections of antistreptococcus serums.

METHODS EMPLOYED.

In the studies now reported guinea-pigs have been employed exclusively for the tests because of the relative ease with which they are secured in sufficient number and of a uniform size. The streptococcus used is designated "Streptococcus S"; it had been rendered quite virulent for guinea-pigs by multiple passages. The minimum lethal dose of a 24-hour broth culture of this organism for medium sized guinea-pigs is from 0.02 to 0.03 c.c. The culture is kept

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[1] "Preliminary Report of Investigations of Serums and Vaccines for Streptococcus, Staphylococcus, and Pneumococcus Infections," *Jour. Am. Med. Assn.*, 1910, 54, p. 257.

on blood agar and its virulence remained unaltered during the time covered by our experiments. At intervals of about a month the stock culture is transferred to fresh slants of blood agar and, after being allowed to grow for 24 hours at 35° C., kept in the ice-box. The fresh cultures used in the experiments were secured by inoculations from such stock cultures. The broth employed was made sugar-free by Theobald Smith's method, and 0.2 per cent of dextrose was added to it before sterilization.

The opsonic determinations have been made with a modified Wright's technic which after long use yields very constant results with slight degree of error. Estimation of the opsonin by dilution to the point of opsonic extinction was also employed. The virulent "Streptococcus S" was used in these tests, and undergoes very little phagocytosis when treated with normal serum. Human leukocytes were used in all cases unless otherwise stated.

We take this opportunity of expressing our obligation to Dr. E. M. Houghton, director of the Biological and Research Departments of Parke, Davis & Co., for supplying us with fresh polyvalent antistreptococcus serums free from preservatives. Serums from three individual horses were sent, packed in ice, each month as soon as possible after the bleedings. These serums have been largely used in our study and are designated by the numbers 891, 893, and 1,130. Unless otherwise stated they contain no preservative.

EXAMINATION OF THE SERUMS OF INDIVIDUAL HORSES.

The serums of the three horses obtained at four successive monthly bleedings were examined for opsonin five to 10 days after being drawn. The amount of opsonin at this primary examination as determined by the usual method and by dilution to the point of opsonic extinction is shown in Table 1. The results secured by the two methods of estimation correspond quite closely. It will be noted that the amount varies considerably among the horses and also in the same horses at different bleedings. Some of the serums had been kept longer than others after being drawn because of delay in transportation, so that the variation may be greater than would be the case if the examinations had been made immediately after the blood was drawn.

TABLE 1.*

SHOWING THE OPSONIC POWER OF THE SERUMS OF THREE HORSES SOON AFTER BLEEDING IN FOUR SUCCESSIVE MONTHS.

Date of Bleeding	No. of Horse	No. of Leukocytes out of 50 Taking Part	Average No. of Cocci per Phagocyte	Point of Opsonic Extinction
October, 1910	891	29	4.3	1:768
	893	12	1.7	1:6
	1,130	23	4.2	1:192
November, 1910	891	22	3.2	1:768
	893	11	0.8	1:24
	1,130	25	3.9	1:768
December, 1910	891	15	1.4	1:192
	893	9	0.7	1:24
	1,130	1	0.4	1:12
Jaunary, 1911	891	24	4.0	1:384
	893	26	4.1	1:768
	1,130	25	4.4	1:384

* The amount of phagocytosis with normal horse serum was almost nil, scarcely more than with salt solution.

October serums were examined 6 days after bleeding.
November " " " 8 " " "
December " " " 10 " " "
January " " " 6 " " "

PERSISTENCE OF OPSONIC POWER.

At intervals of about two weeks the examinations were repeated. It was soon evident that the opsonic power progressively and rapidly disappeared from serums kept in the ice-box, and at the end of a month all the serums had lost their property of opsonizing virulent streptococci. The rapidity with which this occurred in

TABLE 2.*

SHOWING THE PERIODS DURING WHICH THE SERUMS FROM THREE HORSES IN FOUR SUCCESSIVE MONTHS RETAINED THEIR OPSONIC ACTIVITY AND THE PERIOD DURING WHICH THE SAME SERUMS COULD BE REACTIVATED BY FRESH SERUM.

Date of Bleeding	No. of Horse	Point of Opsonic Extinction When First Examined	Latest Day after the Bleeding When the Opsonic Power Was Demonstrated in Serum	Latest Time after Bleeding When Reactivation Was Accomplished by Adding Fresh Normal Human Serum
October, 1910	891	1:768	20 days	29 days
	893	1:6	15 "	21 "
	1,130	1:192	30 "	4 months
November, 1910	891	1:768	13 days	28 days
	893	1:24	9 "	0 "
	1,130	1:768	21 "	4 months+
December, 1910	891	1:192	27 "	2 months
	893	1:24	20 "	58 days
	1,130	1:12	27 "	2 months
January, 1911	891	1:384	13 days	45 days
	893	1:768	13 "	24 "
	1,130	1:384	24 "	2 months+

* October serums were first examined 6 days after bleeding.
November " " " " 8 " " "
December " " " " 10 " " "
January " " " " 6 " " "

the various serums is shown in Table 2. It is fair to infer that considerable loss in opsonic power occurred during the six to 10 days immediately following the drawing of the bloods, and that the serums which were examined longest after the bleeding had lost relatively most. This would account for the smaller opsonic content of the serums which were primarily examined from two to four days later than the other two sets.

THE REACTIVATION OF SERUM.

When the serums had lost their opsonic power as tested in the manner indicated, efforts were made to restore their opsonizing power by the addition of fresh normal serum, human and guinea-pig. The serum of the guinea-pig was chosen because it was the animal used in testing the protective power of the serums, and human serum because antistreptococcus serums are employed therapeutically in man. In these experiments nine parts of fresh normal serum were added to one part of the serum to be tested. It was found that soon after the opsonic power disappeared from the serums, an opsonic activity almost equal to the original could be secured by the addition of fresh normal serum. For some reason the serum from horse 1,130 retained its property of being reactivated by normal serum longer than did the serums of the other horses (see Table 2).

The property of reactivating antistreptococcus serum is possessed in a higher degree by fresh normal human serum than by fresh guinea-pig serum. In the subjoined experiment reactivation of the immune serum was accomplished by human serum when the immune serum in the mixture was to the normal serum as 1:160, while activation by guinea-pig serum occurred only when the proportion was as 1:10 (see Table 3).

NATURE OF ANTIBODIES.

Heating antistreptococcus serums to 60° C. for one-half hour does not affect their subsequent reactivation by fresh normal serums, but fresh human or guinea-pig serums heated to 60° for one-half hour have lost their power of reactivating old antistreptococcus serums. This appears to point to the complement of the fresh

serum as the essential factor in the reactivation. Aronson[1] has pointed out that the antibodies are of the class of Ehrlich's amboceptors, withstanding 62°–63° for one hour and not being injured by 0.4 per cent trikresol.

TABLE 3.

Antistreptococcus Serum	Salt Solution	Fresh Normal Human Serum	Fresh Normal Guinea-Pig Serum	Average No. of Cocci per Phagocyte
0.0	0.1	0.9	...	0.64
0.1	0.0	0.9	...	2.1
0.05	0.05	0.9	...	1.9
0.025	0.075	0.9	...	2.6
0.0125	0.0875	0.9	...	1.5
0.00625	0.09375	0.9	...	2.2
0.003125	0.096875	0.9	...	0.7
0.0	0.1	...	0.9	0.6
0.1	0.0	...	0.9	1.24
0.05	0.05	...	0.9	0.4
0.025	0.075	...	0.9	0.7

As in the case of diphtheria antitoxin, the antistreptococcus bodies are closely associated with the pseudoglobulins, as shown by the following experiment: 1,950 c.c. of antistreptococcus serum was precipitated with 2,385 c.c. of a saturated solution of ammonium sulphate, a proportion of 9:11 or a saturation of 55 per cent. The precipitate was pressed out, redissolved in water and the solution saturated with sodium chloride. This solution was filtered and the globulins precipitated by the addition of 2.75 c.c. acetic acid per liter. This precipitate was pressed out, dialyzed, neutralized, and filtered through a Berkefeld filter.[2] The material thus obtained was tested as to its opsonic activity when activated by fresh serum, and as to its protective power in guinea-pigs. When mixed with fresh serum in the proportion of nine to one, the point of opsonic extinction was reached at a dilution of 1:768 in the case of concentrated serum, as compared with 1:192 in the case of the original serum.

In guinea-pigs 0.5 c.c. of the concentrated serum protected against one M.L.D. of living culture, while one c.c. of the original serum was required to accomplish this. It remains to be determined whether the antistreptococcus bodies can be secured in

[1] "Untersuchungen über Streptokokken u. Antistreptokokken-Serum," *Berl. klin. Wchnschr.*, 1902, 39, pp. 979, 1006.

[2] This concentration was made by P. G. Heinemann of the University of Chicago.

greater concentration by employing the methods elaborated by Banzhof for the concentration of diphtheria antitoxin.

EFFECTS OF TRIKRESOL ON PHAGOCYTOSIS.

If we undertake the estimation of the opsonic content of serums containing trikresol by the usual method we may obtain erroneous results because of the inhibiting action of the preservative on the leukocytes. That the action of the preservative is on the leukocytes and not on opsonin is shown by the fact that a non-virulent streptococcus suspended in salt solution in the absence of serum is taken up by leukocytes to a considerable extent, while if 0.3 per cent trikresol is added phagocytosis ceases. Nine parts of a 0.3 per cent solution of trikresol in physiologic salt solution added to one part of active serum inhibits phagocytosis, while one part of the former solution added to nine parts of active serum does not. The exact concentration of trikresol required to check phagocytosis lies about midway between these two points.

The influence of trikresol in the estimation of opsonic power in the usual way is shown in the following results obtained with three fresh antistreptococcus serums with and without the addition of trikresol:

891	—without trikresol = phagocytic index	4.8
893	— " " = " "	2.6
1,130	— " " = " "	3.0
891	—with 0.4 per cent trikresol = phagocytic index	0.0
893	— " 0.4 " " = " "	0.0
1,130	— " 0.4 " " = " "	0.0

Hence in estimating the opsonic power of serums containing trikresol they must first be diluted to a point where the preservative becomes inactive. If the method of dilution to the point of opsonic extinction is used this source of error is eliminated.

In estimating the degree to which antistreptococcus serums may be reactivated by fresh serum the influence of preservatives must also be remembered. In the following experiment 0.4 per cent trikresol was added to an old antistreptococcus serum just before making the mixtures. The possible error in estimating the degree of reactivation by the usual method is apparent.

TABLE 4.

	Normal Fresh Serum	Leukocytic Suspension	Strepto-coccus Sus-pension	Average Bacteria per Phago-cyte	Number of Cells in 50 Taking Part
891 without trikresol, 9 parts	1 part	1 part	1 part	4.6	23
893 " " 9 parts	1 part	1 part	1 part	1.2	10
1,130 " " 9 parts	1 part	1 part	1 part	4.3	23
891 with trikresol, 9 parts	1 part	1 part	1 part	0.06	2
893 " " 9 parts	1 part	1 part	1 part	0.06	2
1,130 " " 9 parts	1 part	1 part	1 part	0.0	0

PROTECTIVE ACTION IN GUINEA-PIGS.

The serums from the three horses were tested for three successive months, as soon as possible after being received, for their power to protect guinea-pigs against the virulent "Streptococcus S." The serum in varying doses was injected subcutaneously and on the following day one M.L.D. of the culture was injected intraperitoneally. This method was chosen to allow the removal and examination of the peritoneal exudate at intervals after the injection. The dose of the 24-hour broth culture required to kill all guinea-pigs from 250 to 350 gms. in weight varied from 0.02 to 0.03 c.c. In most tests 0.025 c.c was the amount injected.

The amount of serum required to protect against such a dose of streptococci varied among the horses and in the same horse at different months. In most instances seven c.c. of serum gave perfect protection, several times three c.c. were sufficient and in a few cases one c.c. was enough. No protection was secured with less than one c.c. of any serum. Even with an excess of serum the dose of culture could not be increased appreciably without breaking down the protection. The number of cocci contained in the M.L.D. seems to be a factor of importance, only a certain number being disposed of by the leukocytes which migrate into the peritoneal cavity. If the number of cocci injected is so large that they are able to multiply before there are enough leukocytes to take them up, the infection progresses and usually ends fatally. Guinea-pigs which have been perfectly protected against the injected streptococci have no free cocci in the peritoneal cavity after 24 hours. Often none can be found after four or five hours. In guinea-pigs which were partially protected the cocci were not entirely destroyed

at once, and those remaining free multiplied and were only disposed of after two to four days. These animals behave like over-resistant normal ones, the injected serum placing them in a condition to overcome the infection but not affording complete protection.

The results obtained through examination of peritoneal exudates in injected animals have corresponded to those obtained and described by Bordet[1] in his classical paper on this subject.

In perfectly protected animals there was a prompt and abundant migration of leukocytes accompanied by rapid phagocytosis of the cocci. The cocci were soon destroyed in the cells, first staining poorly and then disappearing entirely. At the end of 24 hours no free cocci were seen, and often in four hours few could be seen even in the phagocytes. When the bacteria had been disposed of abundant endothelial cells appeared in the exudate and they in turn took up the leukocytes. In partially protected and very resistant normal animals the bacteria were taken up in part by the leukocytes which were less abundant than in the former case. The free bacteria multiply and the final result as to the fate of the animal depended on whether the bacteria or leukocytes finally obtained the upper hand. So long as there were many free cocci and much phagocytosis by leukocytes, there was no considerable increase of endothelial cells, which appear to perform their services after the struggle between the leukocytes and bacteria is over. In unprotected animals, and in most normal ones the bacteria multiplied rapidly, there were few leukocytes and little phagocytosis, and at the time of death the exudate contained few leukocytes and enormous numbers of free streptococci.

The character of the peritoneal exudate in individual animals is briefly shown in Tables 7 and 8.

The protective power of the serums in guinea-pigs corresponded with the ability of being reactivated by fresh serum. Serums which at first protected the animal against the living culture of streptococci lost this property when they were no longer capable of being reactivated. We have tested the activity of reactivated antistreptococcus serum upon different streptococci, and have observed nothing which would point toward any selective action on any of the different strains of streptococci used.

[1] "Contribution à l'étude du sérum antistreptococcique," *Ann. de l'Inst. Pasteur*, 1897, 11, p. 177.

ALTERATIONS IN LEUKOCYTES AND OPSONIN IN INJECTED GUINEA-PIGS.

Guinea-pigs received subscutaneously six c.c. of an old antistreptococcus serum, being a dose slightly in excess of that required to protect against one M.L.D. of virulent streptococci injected into the peritoneal cavity 24 hours later. Such animals were examined at intervals as to the number and activity of the leukocytes in the peripheral blood, and as to alterations in the opsonic power of the serum for virulent streptococci. No increase in leukocytes was observed. There was an increased phagocytic activity of the leukocytes which disappeared 24 to 48 hours after the injection. This increased leukocytic activity appears to be specific for streptococci. The opsonic power of the serum of circulating blood for streptococci was increased, beginning one hour after injection of the serum and persisting for about 10 days. Table 5 shows the results obtained in such an experiment.

TABLE 5.

	Before Injection	Interval after Injection											
		1 Hour	2 Hours	3 Hours	4 Hours	16 Hours	2 Days	3 Day	4 Days	6 Days	8 Days	10 Days	16 Days
Opsonic index	0.5	0.9	1.3	1.2	1.3	2.1	2.3	2.3	3.2	2.4	1.5	1.6	0.4
Phagocytic activity of leukocytes as compared with normal...........	1.0	...	...	...	...	2.1	2.5	0.7	0.7	...	...	...	...

In animals injected with corresponding amounts of normal horse serum, no such alterations in opsonin and leukocytic activity were observed.

When the antistreptococcus serum is injected into the peritoneal cavity there follows a leukocytosis, associated with alterations in opsonic and leukocytic activity similar to those following subcutaneous injections.

DURATION OF PROTECTION.

With the object of learning the duration of the protection afforded by antistreptococcus serum, a series of guinea-pigs were each injected with six c.c. of an active serum subcutaneously.

After varying intervals they received one M.L.D. of culture intraperitoneally. The protection was found to persist for about eight days (Table 6).

This corresponds very closely with the period during which an elevation of opsonic power was demonstrable in the blood of animals which had received the serum.

TABLE 6.

POLYVALENT ANTISTREPTOCOCCUS SERUM FOLLOWED BY ONE M.L.D. OF LIVING STREPTOCOCCUS CULTURE.

Pig No.	Weight in Grams	Antistreptococcus Serum—1,130—Subcutaneously	"Streptococcus S," 24-Hour Broth Culture—Intraperitoneally	Interval between the Two Injections	Result
1	250	6 c.c.	0.025 c.c.	0	Died
2	255	"	"	1 hour	"
3	265	"	"	2 hours	"
4	270	"	"	3 "	"
5	300	"	"	4 "	Survived
6	250	"	"	17 "	"
7	280	"	"	1 day	"
8	270	"	"	2 days	"
9	250	"	"	3 "	"
10	260	"	"	4 "	"
11	260	"	"	5 "	"
12	250	"	"	6 "	"
13	265	"	"	. "	"
14	270	"	"	10 "	Died
15	290	"	"	12 "	"

COMMERCIAL ANTISTREPTOCOCCUS SERUMS.

The methods of study which we have related as applied to the serums of individual horses were also used in the study of five commercial antistreptococcus serums; three of American and two of European manufacture. A résumé of the results secured with the American serums is shown in Table 7. Serum P (Parke, Davis & Co.) afforded complete protection against one M.L.D. of the culture in doses of two and six c.c., serum M (H. K. Mulford Co.) in doses of one, two, and six c.c., serum X had little protective power, the test animals corresponding quite closely to the control. Serum M did not protect in doses of less than one c.c. In this as in other tests normal horse serum appeared to lower the resistance, or it at least had no protective power.

Of the two antistreptococcus serums of European manufacture examined, one (Aronson's 20-fold) protected against one M.L.D. of the cultures in doses of one to six c.c., and the other (Institut Pas-

TABLE 7.

Guinea-Pigs	Weight in Grams	Commercial Antistreptococcus Serums—Subcutaneously Feb. 13, 1911 4 P.M.			Normal Horse Serum—Subcutaneously Feb. 13, 1911, 4 P.M.	"Streptococcus S," 24-Hr. Broth Culture—Intraperitoneally Feb. 14, 1911, 5 P.M.	Antistreptococcus Serums Reactivated with Normal Fresh Human Serum 1 Part Immune to Normal 9 Parts Opsonin			Opsonin in Serum of Guinea-Pigs				February 15, 9:30 A.M.		February 16, 9 A.M.		February 17, 10 A.M.		February 18, 9 A.M.	Fate of Pigs
										Before Serum Was Injected		24 Hrs. after Serum Was Injected									
		P	M	X			No. of Leukocytes out of 50 Taking Part	Average No. of Cocci per Phagocyte	Point of Opsonic Extinction	No. of Leukocytes out of 50 Taking Part	Average No. of Cocci per Phagocyte	No. of Leukocytes out of 50 Taking Part	Average No. of Cocci per Phagocyte	General Condition	Peritoneal Exudate	General Condition	Peritoneal Exudate	General Condition	Peritoneal Exudate		
1...	240	1 c.c.	.....	.....	.....	0.02 c.c.	21	4.2	1:384	1	0.04	8	1.8	Very sick	F.C., +++ L., ++ P., ++ E., o	Dead	....	....	....	.....	Died
2...	260	2 c.c.	....	.....	....	0.02 c.c.	..	.....	.....	3	0.1	11	1.5	Lively	F.C., o L., ++ P., o E., +	Lively	F.C., o L., +++ P., o E., ++	Lively	F.C., o L., + P., o E., +++	Lively	Survived
3...	280	6 c.c.	....	.....	.....	0.02 c.c.	..	....	.....	4	0.2	10	0.8	Lively	F.C., o L., ++++ P., + E., ++	Lively	F.C., o L., +++ P., o E., ++	Lively	F.C., o L., + P., o E., +++	Lively	Survived
4...	240	.....	1 c.c.	.....	.....	0.02 c.c.	28	2.9	1:384	0	0.0	2	0.1	Lively	F.C., o L., ++++ P., o E., ++	Lively	F.C., o L., +++ P., o E., ++	Lively	F.C., o L., + P., o E., +++	Lively	Survived
5...	265	.....	2 c.c.	.....	.....	0.02 c.c.	....	....	.....	1	0.06	7	1.4	Lively	F.C., o L., ++++ P., o E., ++	Lively	F.C., o L., +++ P., o E., ++	Lively	F.C., o L., + P., o E., +++	Lively	Survived

6..	290	.....	6 c.c.	.....	.....	0.02 c.c.	....	....	.....	0	0.0	6	1.6	Lively	F.C.. o L., +++ P., + E., +	Lively	F.C., o L., +++ P., + E., ++	Lively	F.C., o L., +++ P., o E., +++	Lively	Survived
7..	250	.....	.....	1 c.c.	.....	0.02 c.c.	7	0.98	1:48	1	0.06	1	0.08	Sick	F.C., ++ L., ++ P., + E., o	Fairly lively	F.C., + L., ++++ P., ++ E., +	Fairly lively	F.C., + L., ++ P., + E., ++	Lively	Survived
8..	270	.....	.....	2 c.c.	.....	0.02 c.c.	....	....	.....	2	0.16	2	0.1	Sick	F.C., ++ L., +++ P., + E., o	Fairly lively	F.C., + L., ++ P., ++ E., +	Fairly lively	F.C., o L., ++++ P., + E., ++	Lively	Survived
9..	315	.....	.....	6 c.c.	.....	0.02 c.c.	....	....	.....	0	0.0	5	0.8	Slightly sick	F.C., ++ L., +++ P., + E., o	Fairly lively	F.C., + L., +++ P., + E., +	Fairly lively	F.C., o L., +++ P., o E., ++	Lively	Survived
10..	300	.....	.....	.....	6 c.c.	0.02 c.c.	9	0.8	1:96	0	0.0	3	0.9	Very sick	F.C., +++ L., ++ P., ++ E., o	Dead	....	....	....	.....	Died
11..	320	.....	.....	.....	.....	0.02 c.c.	....	....	.....	0	0.0	0	0.0	Sick	F.C., ++ L., ++ P., ++ E., o	Fairly lively	F.C., + L., +++ P., + E., +	Fairly lively	F.C., + L., +++ P., + E., ++	Lively	Survived

Tests of commercial antistreptococcus serums: F.C. = free cocci; L. = leukocytes; P. = phagocytosis; E. = endothelium.

teur) in doses of two to six c.c. Smaller doses were without effect. It is thus apparent that the two active American serums possessed a protective power equal to that of the European serums.

Our former failures to secure any protection with commercial antistreptococcus serums, it is now believed, may have been due to lack of appreciation of the importance of the relation between the degree of immunity and the number of bacteria injected.

It is not unlikely that the number of bacteria contained in a M.L.D. of the culture is an important factor in determining the amount of serum required for protection. We have estimated by dilution and plate cultures that one M.L.D. of our culture contains about 12,500,000 cocci. Of the culture used by Bordet in his test of the action of antistreptococcus serums in rabbits, the M.L.D. intraperitoneally was 0.000,001 c.c. or approximately 500 cocci. The culture employed by Aronson in standardizing his antistreptococcus serum in mice possesses a M.L.D. of 0.000,000,05 c.c. or approximately five cocci. Since protection is apparently dependent on rapid and complete destruction of the cocci after injection, it appears reasonable that with an equal quantity of available immune bodies, opsonification and subsequent phagocytic destruction would be more likely if very few cocci are present. For this reason the most highly virulent cultures would seem to be the best suited for measuring comparatively low degrees of immunity. With such cultures in susceptible animals, 10 or 100 times the M.L.D. contains relatively few cocci.

It is of interest to note that the serums which were reactivated to a considerable degree were also the ones which afforded protection to the animals. In the animals which showed complete protection the opsonic power in the blood rose after the injection of the serum, while in the others this did not occur except in one case (guinea-pig 1).

OLD COMMERCIAL SERUM.

Five commercial antistreptococcus serums which had been in the ice-box for over two years and whose dates for use had expired were tested in a similar manner. The results are given in Table 8.

TABLE 8.

Guinea-Pigs	Weight in Grams	Old Antistreptococcus Serum—Subcutaneously Feb. 27, 1911, 5 P.M.					Old Normal Horse Serum—Subcutaneously Feb. 27, 1911, 5:00 P.M.	"Streptococcus S." 24-Hr. Broth Culture—Intraperitoneally Feb. 28, 1911, 3:30 P.M.	Point of Opsonic Extinction of Serums Reactivated with Normal Human Serum; 1 Immune to 9 Normal	Opsonic Index of Serum of Guinea-Pigs		March 1, 9 A.M.		March 2, 9 A.M.		Fate of Pigs
		A	H	M	S	C				Before Serum Was Injected	18 Hrs. after Serum Was Injected	General Condition	Peritoneal Exudate	General Condition	Peritoneal Exudate	
1	250	6 c.c.	.....	.....	.....	.....	.....	0.025 c.c.	1:1536	.3	1.4	Lively	F.C., o L., ++++ P., o E., +++	Lively	F.C., o L., + P., o E., +++	Survived
2	250	.....	6 c.c.	.....	.....	.....	.....	0.025 c.c.	1:48	1.0	0.9	Very sick	F.C., ++++ L., ++ P., +++ E.,	Dead	....	Died
3	250	.....	.....	6 c.c.	.....	.....	.....	0.025 c.c.	1:384	.3	1.7	Lively	F.C., o L., ++++ P., + E., ++	Lively	F.C., o L., + P., o E., +++	Survived
4	250	.....	.....	.....	6 c.c.	.....	.....	0.025 c.c.	1:768	.3	1.6	Lively	F.C., o L., ++++ P., o E., +++	Lively	F.C., o L., ++ P., o E., +++	Survived
5	260	.....	.....	.....	.....	6 c.c.	.....	0.025 c.c.	1:48	.3	1.5	Quite sick	F.C., ++ L., ++ P., + E.	Very sick	F.C., ++ L., ++ P., + E.	Died
6	220	.....	.....	.....	.....	.....	6 c.c.	0.025 c.c.	.....	1.0	1.0	Very sick	F.C., +++ L., +++ P., +++ E.	Dead	....	Died
7	255	.....	.....	.....	.....	.....	.....	0.025 c.c.	.....	...	...	Quite sick	F.C., ++ L., +++ P., ++ E.	Quite sick	F.C., + L., ++ P., +++ E.	Survived

Tests of antistreptococcus serums over two year old: F.C. = free cocci; L. = leukocytes; P. = phogocytosis; E = endothelium.

CURATIVE POWER IN GUINEA-PIGS.

We have been unable to observe any curative effects from serums given after the injection of the cultures, and if the serums were given less than four hours before the culture no protection was manifest.

The rapidity with which a streptococcus infection in guinea-pigs runs its course and terminates in death or recovery makes it difficult to study in this animal the curative power of antistreptococcus serum. The curative effects described in animals as related by several workers have been secured only if the serum was given very soon after the infection had been produced and not later than a few hours.

PROTECTIVE AND CURATIVE ACTION ON MAN.

We have pointed out that the antistreptococcus serums are reactivated more readily and to a greater degree by fresh human that by fresh guinea-pig serum. This may explain the relatively large dose of serum required to protect the guinea-pig and may indicate a relatively greater protective and curative power in man from the same serum.

The ability of fresh blood serum to reactivate an antistreptococcus serum appears to furnish quite an accurate index to the probably protective activity of the serum when injected in the guinea-pig. It may be inferred that this will also hold good in man and that individuals may be protected from subsequent streptococcus infections by the injections of suitable quantities of antistreptoccus serum.

Determination of the opsonic content of the blood of the injected individual may serve to indicate when enough serum has been given to produce protection or to have a curative effect, as in guinea-pigs protected in this way the opsonin in the blood serum was appreciably increased. Since reactivation of antistreptococcus serum by fresh human serum is not obtained at a very high dilution, it probably follows that the efficient dose in man must be rather large. This is in accord with the experience of those who have secured favorable results in the clinical use of the serum.

It is very difficult to compare the therapeutic effects of antistreptococcus serum in animals with their rapidly fatal generalized

infections with those in man with the usually more prolonged and localized disease.

In cases of streptococcus infection in man we have several times found that the fresh serum of the individual is still able to reactivate antistreptococcus horse serum, even though the patient's blood contains much less opsonin for streptococci than normal. This would suggest that the immune serum would be active against the infection.

In normal guinea-pigs the injection of antistreptococcus serum has a direct stimulating effect on the phagocytic activity of the leukocytes. In some cases of streptococcus infection in man the leukocytes are reduced in their phagocytic power, and are relatively inactive in the presence of opsonin. It would be of interest to know whether the antistreptococcus serum may be useful in restoring the activity of the leukocytes in such cases.

STANDARDIZATION OF ANTISTREPTOCOCCUS SERUM NEEDED.

In view of the fact that one of the three antistreptococcus serums which were tested was almost entirely lacking in any immune bodies as indicated by the animal tests and also by the reactivation experiments, it would seem desirable to have some guarantee that such serums when offered for sale are active. If possible a standard should be established by the Hygienic Laboratory, United States Public Health and Marine Hospital Service. We believe that estimations of the reactivability of immune serums by fresh serum using a virulent culture in making the determinations would be a distinct aid in such standardization. Whether this method could be also applied to such serums as that of Moser, in the preparation of which cultures non-virulent for animals are employed, we have not had an opportunity of learning.

CONCLUSIONS.

Antistreptococcus serums rapidly lose their opsonic power, which may for some time be largely restored by the addition of fresh human or guinea-pig serum.

Guinea-pigs may be protected against virulent cultures of streptococci by previous injection of antistreptococcus serums.

The protective power of immune serums continues so long as they can be reactivated by fresh serum.

Injections of immune serums in guinea-pigs may be followed by increased activity of leukocytes of short duration and by an increased opsonic power for streptococci in the blood serum persisting for about 10 days.

The immunity in guinea-pigs produced by injection of immune serum persists for about eight days.

Of three fresh commercial serums manufactured in the United States which were tested two were active and one inactive. The two European serums were active.

Fresh normal human serum and fresh human serum from persons infected with streptococci are able to reactivate antistreptococcus serums. This indicates that such serums may have some protective and curative effect in man in cases of streptococcus infections.

The specific antistreptococcus bodies are resistant to heat and chemicals (trikresol, 0.4 per cent chloroform) and are closely associated with the pseudoglobulins of the immune serum.

It would be desirable to have some guarantee of the activity of antistreptococcus serums offered for sale.

STUDIES ON THE CHEMISTRY OF ANAPHYLAXIS (III).* EXPERIMENTS WITH ISOLATED PROTEINS, ESPECIALLY THOSE OF THE HEN'S EGG.†

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In the early history of immunology the few preliminary attempts to secure precipitins differentiating different proteins coming from the same animal were so generally unsuccessful that, in spite of some more or less positive results, the opinion became general that the specificity of proteins depends upon certain groups or radicals which are characteristic of the origin of the protein, and perhaps not associated with demonstrable chemical differences. With extension of the investigations to a more varied class of materials, and with refinements of the methods, the accuracy of this hypothesis became more and more questionable, until within the past few years it has come to be recognized that two different proteins from one and the same animal may, in certain cases, be distinguishable by means of the biological reactions. We now consider that a protein may exhibit a specificity characterizing its biological origin, which presumably depends upon certain hypothetical groups common to the animals of a particular origin, and that at least some proteins possess groups which are independent of their origin, and are in characteristic relation to the chemical composition of the proteins. On the one hand it has been shown that the precipitins for one protein of an animal may not react with another protein of the same animal, this having been demonstrated with milk proteins against serum proteins, serum proteins against hemoglobin, lens proteins against serum and tissue proteins. In these cases apparently common species groups are absent. On the other hand we find that antibodies for proteins of one species may react with corresponding proteins of other species; e.g., antibodies for lens proteins of one species will react with lens proteins of an entirely

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† The preceding articles of this series were published as follows: I, this *Journal*, 1908, 5, pp. 449–83; II, *ibid.*, 1909, 6, pp. 506–22.

different species, when they do not react with the serum or tissue proteins of homologous species. Antisera for casein are also said to react much more strongly with casein of foreign species than with lactalbumin or serum albumin of the same species (Bauer). Amyloid obtained from different species of animals reacts with antiserum obtained by immunizing with any sort of amyloid, but antiamyloid serum will not react with the serum of the animal from which the amyloid is derived (Raubitschek[1]). Here we seem to have proteins of similar chemical nature which do not contain the antigen groups characteristic of most of the other proteins of the same animal body.

There still remain, however, many unanswered questions concerning the possibility of distinguishing by different biological reactions proteins of a single animal or plant, even when these proteins are distinctly different from one another according to chemical criteria; that is, we are still far from having successfully correlated the biological and chemical characters of proteins. As most of the work along this line has been done by means of the precipitin reaction, and as the anaphylaxis reaction possesses certain differences, especially as to the possibility of extremely minute quantities of the proteins causing a reaction in animals, it seemed desirable to investigate this problem as far as feasible by means of the anaphylaxis reaction, and thus, if possible, throw upon the field a new light from a different angle. Review of the literature also reveals much confusion and contradiction because of the use of unsuitable methods of separation of proteins, whereby oftentimes mixtures were assumed to be pure proteins, and in other cases chemical modifications of the proteins were unwittingly produced during purification, with misleading results. It does not seem necessary to give here a complete résumé of all this literature, but attention will be directed chiefly to the work which has been done with egg proteins. These were used for most of the experiments to be recorded in this article, because they seemed to offer an especially favorable material. In the first place in egg white we have a protein, *ovomucoid*, which cannot be coagulated by heat and is not irreversibly coagulated by alcohol, and thus it can be

[1] Raubitschek, *Verhandl. Deut. Path. Gesell.*, 1910, 14, p. 273.

separated readily and completely from the other proteins of the egg white; second, the existence of two different parts, the yolk and the white, provides variety of material from a common source; third, at least one of the proteins of egg white can be readily crystallized and recrystallized; and fourth, the egg proteins have received exceptionally thorough chemical investigation.

It has long been recognized that egg white shows a less marked specificity than serum proteins, both as regards precipitin and anaphylaxis reactions. Thus in the early days of precipitin studies Myers[1] observed that precipitins resulting from immunizing with crystallized albumin from fresh hen's eggs reacted slightly with unpurified duck egg white, but not with purified albumin from sheep and bullock blood. Uhlenhuth[2] also found that antiserum for hen egg reacts with pigeon egg white, and conversely, but in each case the reaction was less marked than with the homologous egg white. Rosenau and Anderson[3] in one of their early papers on anaphylaxis reported a series of experiments on guinea-pigs with the whites of eggs of several species, which, as summarized below, exhibited a very irregular and uncertain individuality.

Sensitized	with	hen	egg	white	reacted	strongly	with	crane	egg	white
"	"	"	"	"	"	little	"	duck	"	"
"	"	duck	"	"	"	fatally	"	hen	"	"
"	"	guinea-hen	"	"	"	"	"	"	"	"
"	"	" "	"	"	"	strongly	"	crane	"	"
"	"	pigeon	"	"	"	"	"	"	"	"
"	"	"	"	"	"	negatively	"	hen	"	"
"	"	goose	"	"	"	strongly	"	"	"	"
"	"	"	"	"	"	"	"	turkey	"	"
"	"	turkey	"	"	"	fatally	"	hen	"	"
"	"	"	"	"	"	strongly	"	crane	"	"

These and similar atypical and indefinite results are explained by the recent studies of Welsh and Chapman,[4] who, applying the saturation method to precipitating sera, demonstrate that antiserum for any egg white contains a general avian antisubstance, so that common precipitin reactions are obtained with all bird egg proteins, and also the antiserum contains an independent antisubstance specific for the species. Therefore by quantitative methods it can be found that a greater amount of precipitate is produced with the homologous antiserum, which reacts with both antigens, than with a heterologous antiserum which reacts only with the common avian antigen.

As to the relations of the different proteins which exist, or have been supposed to exist, in egg white, the literature contains but few reports. Levene[5] found that antiserum for whole egg white reacts to both albumin and globulin of egg white, as well as to yolk and chicken serum. Umber[6] found that antiglobulin serum and antialbumin serum both precipitate egg white; globulin as purified by him is pre-

[1] *Lancet*, 1900 (ii), p. 98; *Centralbl. f. Bakt.*, 1908, 28, p. 237.

[2] *Deut. med. Wchnschr.*, 1900, 26, p. 734.

[3] *Bull. Hyg. Lab.*, No. 45.

[4] *Jour. of Hyg.*, 1910, 10, p. 177.

[5] *Medical News*, December 21, 1901.

[6] *Berl. klin. Wchnschr.*, 1902, 39, p. 657.

cipitated by both these antisera, while crystallized albumin he found not precipitated by either. Obermeyer and Pick[1] divided the proteins of egg albumin into four portions (ovomucin, dysglobulin, euglobulin, and pseudoglobulin) and found that precipitins for each fraction reacted with all the other fractions, although irregularly and without distinct specificity.

PREPARATION AND PROPERITIES OF MATERIAL USED.

The proteins from the egg white used in these experiments correspond in a way to those ordinarily designated as ovoglobulin, ovalbumin, and ovomucoid; that is, (1) the fraction precipitated on adding ammonium sulphate to half saturation (ovoglobulin); (2) the protein which crystallizes out on adding acetic acid to the half saturated solution (ovalbumin); and (3) the protein which is not coagulated by heat and which dissolves in water after being precipitated with alcohol (ovomucoid). In order to separate the albumin and "globulin" as completely as possible, the following procedure was followed: The precipitate obtained on beating up the whites of perfectly fresh eggs with an equal volume of ammonium sulphate solution was washed with half saturated ammonium sulphate solution, dissolved in water, and reprecipitated by adding an equal volume of saturated ammonium sulphate solution. After repeating this precipitation three times, three more precipitations were made by adding each time somewhat less than enough ammonium sulphate solution to render the concentration one-half saturation; the amount was determined by the degree of precipitation, and was roughly one-third to three-eighths saturation. In this way about one-half the protein in the original precipitate was eliminated and theoretically none of the ovalbumin should have been included with the "globulin" fraction.

To secure ovalbumin as free as possible from "globulin," the procedure was as follows: After crystallizing the ovalbumin three times in the usual manner, it was then precipitated three times with ammonium sulphate, which was added until a precipitate began to form, and this first part of the precipitate was discarded, the albumin in the filtrate being precipitated out with a little more ammonium sulphate. By this means about half the original crystalline protein was eliminated, and only the part less readily precipitated by ammonium sulphate, and presumably most free

[1] *Wien. klin. Rundschau*. 1902, 16, p. 277.

from globulin, was obtained. That is, we have secured proteins representing the extreme ends of what we may call the "precipitation spectrum" of egg white.

Ovomucoid was prepared in the usual way by pouring diluted and beaten egg white into boiling water acidulated with acetic acid, filtering off the coagulated protein, concentrating to a small bulk, precipitating with alcohol, redissolving in water, and reprecipitating the protein with alcohol three to five times. In all, five preparations of ovomucoid were made, which agreed with one another in giving a strong reaction with Millon's reagent, no reaction for tryptophane, a purplish biuret reaction, and containing much reducing carbohydrate which could be split off by boiling with dilute hydrochloric acid. They differed somewhat in solubility, some dissolving easily in water, some requiring a little alkali for quick solution.

The ovomucoid seems to be a definite compound, and the ovalbumin prepared as above specified should also be a pure protein, although it is not fully established that crystallized egg albumin is one rather than a mixture of proteins. As to the first or "globulin" fraction, there is much question, since the study of egg white by Osborne and Campbell.[1] These investigators separated and examined seventeen fractions of the proteins of egg white, and came to the conclusion that egg white contains four definite proteins: (1) Ovomucin, a glycoprotein constituting about seven per cent of the total protein, which has heretofore been regarded as a globulin; (2) ovalbumin, the easily crystallized component, which constitutes about 50 per cent of the proteins, and yields 2 to 2.5 per cent of carbohydrates on hydrolysis with HCl; (3) conalbumin, which is the non-crystallizable fraction obtained after removing the ovalbumin, and which differs from the ovalbumin also in having a different rotatory power and in coagulating at a slightly lower temperature; (4) ovomucoid, which is separated from ovalbumin by fractional precipitation only with great difficulty, but is readily separated by heat coagulation. According to these findings my "globulin" should consist of ovomucin with perhaps some ovalbumin admixed in indefinite

[1] *Jour. Am. Chem. Soc.*, 1900, 22, p. 413.

proportions; my ovalbumin and ovomucoid should be quite pure preparations of these two proteins.

Ovovitellin from the yolk was studied with a preparation obtained from Dr. T. B. Osborne, which represented a sample of this protein which had been quite thoroughly freed from lecithin by alcohol; this process had robbed it of much of its solubility so that only quite dilute solutions could be obtained in 0.1 per cent NaOH, the strongest alkaline solution which it is safe to use for intraperitoneal injections. This protein is a nucleo-albumin, which yields a pseudonuclein on digestion with pepsin.

ANAPHYLAXIS WITH OVOVITELLIN.

Ovovitellin was not found very satisfactory for anaphylaxis experiments, because of its slight solubility in 0.1 per cent NaOH, yet some results of significance were obtained (Table 1). As reported in the previous paper (II), ovovitellin has distinct specificity against the proteins from egg white. This is now again shown in a larger series of animals, as applying not only to crystallized egg albumin, but also to ovomucoid. There was no distinct reaction between the ovovitellin from the hen's egg and that from the egg of the turtle[1] (*Chelona midas*), but the very slight solubility of the latter renders these results something less than conclusive (Table 1, experiments 19–22 and 32–39).

Crude egg white, obtained from fresh eggs, seems to contain either a trace of vitellin, or else something which sensitizes to vitellin, for experiments 1 to 3 show that crude egg white renders guinea-pigs sensitive to ovovitellin, but purified egg albumin does not sensitize to vitellin (experiments 4–9). Ovomucoid and vitellin are also found not to react with one another (experiments 10–13). Although ovovitellin does not cause strong anaphylactic reactions, yet the results in these experiments are distinct enough to demonstrate that this typical egg yolk protein is biologically distinct from two typical proteins of the white, namely, albumin and ovomucoid.

So far as I can find in the literature, purified yolk protein has not previously been studied as to any of its biological reactions.

[1] For this preparation I am indebted to Professor L. B. Mendel.

TABLE I.
OVOVITELLIN.

	Sensitizing Dose	Days' Interval	Second Injection	Results	Subsequent Injections and Remarks
1	0.001 gm. egg albumin, crude	24	0.08 gm. ovovitellin (hen)	Severe symptoms	48 hrs. later reacted fatally to egg albumin
2	0.001 “ “ “ “	24	0.08 “ “ “	Moderate “	48 “ “ “ “ “ “ “
3	0.001 “ “ “ “	24	0.08 “ “ “	“ “	48 “ “ “ “ “ “ “
4	0.01 “ “ “ pure	20	0.05 “ “ “	No “	48 “ “ “ moderately to egg albumin
5	0.002 “ “ “ “	20	0.05 “ “ “	“ “	48 “ “ “ “ “ “ “
6	0.001 “ “ “ “	20	0.05 “ “ “	“ “	48 “ “ “ “ “ “ “
7	0.0002 “ “ “ “	20	0.05 “ “ “	“ “	48 “ “ “ “ “ “ “
8	0.01 “ “ “ “	22	0.05 “ “ “	“ “	
9	0.002 “ “ “ “	22	0.05 “ “ “	“ “	
10	0.01 “ ovomucoid A	20	0.05 “ “ “	Doubtful “	24 “ “ “ severely to ovomucoid
11	0.002 “ “ “	20	0.05 “ “ “	No “	24 “ “ “ slightly “ “
12	0.001 “ “ “	20	0.05 “ “ “	Doubtful “	24 “ “ “ “ “ “
13	0.0002 “ “ “	20	0.05 “ “ “	No “	24 “ “ “ “ “ “
14	0.001 “ egg globulin	31	0.05 “ “ “	Slight “	
15	0.006 “ ovovitellin (hen)	20	0.075 “ egg albumin, pure	Doubtful “	24 “ “ “ “ “ ovovitellin (hen)
16	0.002 “ “ “	20	0.075 “ “ “ “	“ “	
17	0.001 “ “ “	20	0.075 “ “ “ “	“ “	
18	0.0002 “ “ “	20	0.075 “ “ “ “	“ “	24 “ “ “ “ “ “ “
19	0.006 “ “ “	20	ovovitellin (turtle)	No “	24 “ “ “ severely “ “ “
20	0.002 “ “ “	20	“ “	“ “	
21	0.001 “ “ “	20	“ “	“ “	24 “ “ “ moderately to ovovitellin (hen)
22	0.0002 “ “ “	20	“ “	“ “	
23	0.01 “ “ “	28	0.1 “ ovomucoid A	“ “	24 “ “ “ slightly “ “ “
24	0.002 “ “ “	28	0.1 “ “ “	“ “	24 “ “ “ “ “ “ “
25	0.001 “ “ “	28	0.1 “ “ “	“ “	24 “ “ “ “ “ “ “
26	0.005 “ “ “	20	0.1 “ “ “	Doubtful “	24 “ “ “ severely “ “ “
27	0.001 “ “ “	20	0.1 “ “ “	“ “	
28	0.002 “ “ “	20	0.1 “ “ “	“ “	24 “ “ “ moderately “ “ “
29	0.0001 “ “ “	20	0.1 “ “ “	“ “	24 “ “ “ severely “ “ “
30	0.005 “ “ “	19	0.1 “ “ “	No “	48 “ “ “ slightly “ “ “
31	0.001 “ “ “	19	0.1 “ “ “	“ “	48 “ “ “ moderately “ “ “
32	0.01 “ “ (turtle)	21	ovovitellin (hen)	Slight “	24 “ “ “ slightly “ “ (turtle)
33	0.002 “ “ “	21	“ “	Doubtful “	24 “ “ “ “ “ “ “
34	0.001 “ “ “	21	“ “	Slight “	24 “ “ “ “ “ “ “
35	0.0002 “ “ “	21	“ “	Doubtful “	24 “ “ “ “ “ “ “
36	0.010 “ “ “	20	“ “	No “	
37	0.002 “ “ “	20	“ “	“ “	
38	0.001 “ “ “	20	“ “	“ “	
39	0.0002 “ “ “	20	“ “	“ “	

There are a few statements as to precipitins for egg white and egg yolk, but these were made in the early days of immunological study, and apparently there is no evidence of an accurate separation of the two materials, against which separation there are serious mechanical obstacles.

EXPERIMENTS WITH OVOMUCOID.

The five preparations of ovomucoid studied differed somewhat in solubility, and also somewhat in toxicity for sensitized animals, in which respect they were all less active than globulin or albumin, but more active than the vitellin. Their relative toxicity, when given in doses of 0.1 to 0.5 gm. to guinea-pigs which had been sensitized with the homologous ovomucoid in doses from 0.010 to 0.0002 gm., is shown in the following table:

ANAPHYLACTIC ACTIVITY OF OVOMUCOID PREPARATIONS.

Preparation Used	Degree of Reaction					
	None or Doubtful	Slight	Moderate	Severe	Fatal	Total
A	2	7	1	4	0	14
B	3	0	3	0	0	6
C	0	0	2	1	1	4
D	0	1	1	1	2	5
E	0	1	1	3	1	6
Totals	5	9	8	9	4	35

In other experiments it was found that any ovomucoid preparation sensitized against the others as well as against itself. With all five specimens a definite specificity against crystallized egg white was obtained (Table 2), showing not only a distinct chemical specificity for each protein, but also that by repeated crystallization of egg albumin it can be made free from any appreciable amount of ovomucoid. A smaller number of experiments indicated the individuality of ovomucoid as against "globulin" of egg white, as well as against the vitellin of the yolk. In all cases injection of the heterologous protein did not reduce the sensitivity to the homologous protein to any noticeable degree, as shown by positive reactions when the homologous protein was injected after the heterologous.

TABLE 2.
OVOMUCOID.

	Sensitizing Dose	Days' Interval	Second Injection	Results	Subsequent Injections and Remarks
1	.005 gm. ovomucoid A	24	0.1 gm. albumin, cryst.	No symptoms	24 hrs. later reacted severely to ovomucoid A
2	.0005 " " A	24	0.1 " " "	" "	
3	.010 " " A	30	0.1 " " "	" "	24 " " " " " " A
4	.005 " " A	30	0.1 " " "	" "	
5	.001 " " A	30	0.1 " " "		
6	.010 " " B	..	0.1 " " "		
7	.002 " " B	..	0.1 " " "		
8	.001 " " B	..	0.1 " " "		
9	.0002 " " B	..	0.1 " " "		
10	.002 " " C	20	0.1 " " "	No symptoms	
11	.001 " " C	20	0.1 " " "	" "	
12	.002 " " C	20	0.1 " " "	" "	
13	.002 " " C	20	0.1 " " "	Doubtful "	24 hrs. later reacted severely to ovomucoid C
14	.001 " " C	20	0.1 " " "	No "	24 " " " moderately " " C
15	.001 " " C	20	0.1 " " "	" "	
16	.010 " " D	20	0.1 " " "	Doubtful "	7 days " " fatally " " D
17	.002 " " D	20	0.1 " " "	" "	7 " " " " " " D
18	.005 " " E	19	0.1 " " "	" "	
19	.012 " " E	22	0.15 " " "	Slight "	24 hrs. " " moderately " " E
20	.010 " " E	16	0.1 " " "	No "	
21	.002 " " E	16	0.1 " " "	" "	
22	.001 " " E	16	0.1 " " "	" "	
23	.001 " " A	18	0.1 " globulin	" "	
24	.010 " " A	20	0.05 " ovovitellin (hen)	Slight "	24 " " " severely " " A
25	.002 " " A	20	0.05 " " "	No "	24 " " " slightly " " A
26	.001 " " A	20	0.05 " " "	Doubtful "	24 " " " " " " A
27	.0002 " " A	20	0.05 " " "	No "	24 " " " " " " A
28	.001 " egg albumin, cryst.	22	0.1 " ovomucoid A	" "	24 " " " fatally " albumin
29	.001 " " " "	22	0.1 " " A	" "	24 " " " " " "
30	.001 " " " "	28	0.1 " " A	" "	24 " " " " " "
31	.001 " " " "	28	0.1 " " A	Doubtful "	
32	.001 " " " "	28	0.1 " " A	No "	
33	.001 " " " "	22	0.1 " " B	Doubtful "	24 " " " " " "
34	.0001 " " " "	22	0.1 " " B	" "	24 " " " " " "
35	.001 " " " "	24	0.1 " " C	" "	24 " " " " " "
36	.001 " " " "	24	0.1 " " C	No "	
37	.010 " " " "	20	0.1 " " D	" "	
38	.002 " " " "	20	0.1 " " D	" "	
39	.001 " " " "	30	0.2 " " E	" "	
40	.002 " " " "	24	0.2 " " E	" "	24 " " " " " "
41	.001 " " globulin	31	0.1 " " A	" "	24 " " " severely " egg white
42	.001 " " "	31	0.1 " " B	" "	
43	.005 " ovovitellin	20	0.1 " " A	Doubtful "	24 " " " " " ovovitellin
44	.001 " "	20	0.1 " " A	" "	
45	.0005 " "	20	0.1 " " A	" "	24 " " " " " "
46	.0001 " "	20	0.1 " " A	" "	24 " " " moderately " "
47	.002 " "	19	0.1 " " A	No "	48 " " " slightly " "
48	.001 " "	19	0.1 " " A	" "	48 " " " moderately " "

EXPERIMENTS WITH THE "GLOBULIN" FRACTION OF EGG WHITE.

The published evidence as to the anaphylactic activity of the various proteins of serum is altogether contradictory. In studies of anaphylaxis with different fractions of serum, Gay and Adler[1] found that the euglobulin fraction (one-third saturation with ammonium sulphate) sensitizes as well as the entire serum, but when purified is not toxic, while the later fractions of serum proteins are highly toxic but less active in sensitizing. Cabannes,[2] on the other hand, found that the globulins are somewhat more toxic than the serum albumin. Bruynoghe,[3] however, found albumin, euglobulin, and pseudoglobulin all capable of sensitizing and intoxicating, the pseudoglobulin producing the least severe intoxication; while Doerr and Russ[4] state that the globulin alone exhibits either sensitizing or intoxicating properties. Doerr and Russ[5] also found that passive sensitization occurred only with the blood of rabbits immunized with globulin, and not when the immunization was performed with serum albumin.

There seems to be very little difference in activity between the fractions of egg white which correspond in precipitability with the albumin and globulin of serum.

It can be seen from experiments 1–11, Table 3, that the minimum fatal sensitizing dose of the egg "globulin" is very small, 0.000,001 gm. being efficient; fatal intoxication was obtained with 0.001 gm., while 0.0001 gm. caused a slight reaction. These figures are practically identical with those obtained with crystallized albumin, but it should be said that in general minimum doses of "globulin" produced fatal results somewhat less constantly than did corresponding doses of albumin. Therefore, in egg white the two chief proteins are almost equal in their degrees of anaphylactic toxicity, the albumin being slightly the more active of the two.

Experiments 12–26 show that the albumin and globulin fractions react with one another to a very considerable extent. Even very minute doses of one will sensitize a guinea-pig to the other protein, suggesting that these fractions may represent identical proteins.

[1] *Jour. Med. Res.*, 1908, 18, p. 433.

[2] *Compt. Rend. Soc. Biol.*, 1907, 62, p. 809.

[3] *Arch. Internat. Pharmacodynam.*, 1909, 19, p. 393.

[4] *Zeit. Immunität*, 1909, 2, p. 109.

[5] *Ibid.*, 1909, 3, p. 181.

TABLE 3

"GLOBULIN" FRACTION OF EGG WHITE.

	Sensitizing Dose	Days' Interval	Second Injection	Results	Subsequent Injections and Remarks
1........	.0001 gm. egg globulin	26	0.1 gm. egg globulin	Death, 16 min.	
2........	.0001 " " "	26	0.1 " " "	" 25 "	
3........	.00001 " " "	26	0.1 " " "	" 2 "	
4........	.000001 " " "	26	0.1 " " "	" 45 "	
5........	.0000001 " " "	26	0.1 " " "	Slight	
6........	.0001 " " "	20	0.002 " " "	Death, 30 min.	
7........	.0001 " " "	20	0.002 " " "	" 5 "	
8........	.0001 " " "	30	0.001 " " "	" 30 "	
9........	.001 " " "	30	0.0005 " " "	Moderate	3 days later reacted slightly to egg globulin
10........	.0005 " " "	30	0.0005 " " "	Slight	3 " " " fatally " " "
11........	.001 " " "	30	0.0001 " " "	"	3 " " " moderately " " "
12........	.010 " " "	28	0.1 " albumin	Death, 20 min.	
13........	.0001 " " "	28	0.1 " "	" 15 "	
14........	.00005 " " "	28	0.1 " "	" 15 "	
15........	.0005 " " "	21	0.001 " "	Severe	24 hrs. " " " " " "
16........	.0005 " " "	22	0.002 " "	Slight	
17........	.001 " " "	24	0.10 " "	"	
18........	.001 " " "	31	0.10 " "	Death, 10 min.	
19........	.001 " " "	31	0.10 " egg white	" 30 "	
20........	.010 " albumin	28	0.1 " globulin	Moderate	24 " " " fatally " albumin
21........	.0005 " "	28	0.1 " "	"	24 " " " " " "
22........	.0001 " "	28	0.1 " "	"	24 " " " severely " "
23........	.00001 " "	28	0.1 " "	"	24 " " " moderately " "
24........	.001 " "	21	0.001 " "	No symptoms	24 " " " severely " "
25........	.001 " "	22	0.001 " "	Slight	48 " " " fatally " "
26........	.001 " "	24	0.002 " "	Moderate	24 " " " " " "

Against this assumption, however, is the fact that small intoxicating doses (1–2 milligrams) do not usually cause severe reactions with the heterologous sensitization. As the polysensitization experiments (described below) show, there really are two distinct proteins present in these two fractions, but evidently there is either a common protein mixed in both, or else there is a common antigen group in addition to the specific one. Possibly we have three proteins in the two fractions, in the "globulin" fraction being ovomucin and ovalbumin, in the crystallized albumin being ovalbumin and conalbumin or some other, unidentified protein. It is certainly an interesting coincidence, at least, that by the anaphylaxis reaction we find three antigens present in the coagulable part of egg white, and that chemical examination led Osborne and Campbell to distinguish three proteins in the same material.

In studying closely related or mixed proteins by the precipitin test the method of saturation has been found of great value. By adding one protein to an antiserum until all the specific precipitins have been exhausted, the presence of co-existing precipitins specific for a closely related protein then may be demonstrated by adding this protein to the exhausted antiserum. In this way it has been found possible to differentiate between sera of such closely related species as man and monkey, dog and fox, etc. The same principle has also been applied successfully with agglutinins and complement fixators, but, so far as I can learn, not in anaphylaxis. It is equally feasible in anaphylaxis, however, as the experiments in Table 4 demonstrate. If a sensitized animal is given a sufficient quantity of the specific antigen and recovers from the reaction, it will be refractory to another dose of the same antigen given within the next few days. According to Friedberger's hypothesis this phenomenon depends simply upon the exhaustion or saturation of the specific antibody, in favor of which is the fact which I have observed many times, that if the intoxicating dose is very small (1–2 milligrams of egg albumin) even although the animal reacts severely yet it will not usually be rendered refractory by this reaction, presumably because the amount of antigen injected has not combined all the antibody. Evidently it is not the severity of the reaction which determines

TABLE 4.

Reaction of Guinea-Pigs Sensitized to Whole Egg White (0.006 gm.) Three Weeks, to Isolated Egg Proteins Injected at 24 Hour Intervals.

Second Injection	Result	Third Injection	Result	Fourth Injection	Result	Fifth Injection	Result	Sixth Injection	Result
Gm.		Gm.		Gm.		Gm.		Gm.	
1..0.050 globulin	Severe	0.050 globulin	Slight	0.1 albumin	Died in 55 min.				
2..0.050 "	"	0.050 "	None	0.1 "	" " 50 "				
3..0.033 "	"	0.050 "	Slight	0.050 "	Moderate				
4..0.033 "	"	0.050 "	"	0.050 "	"	0.1 whole egg white	Severe		
5..0.033 "	"	0.050 "	"	0.050 "	Severe				
6..0.002 albumin	Slight	0.010 albumin	"	0.050 "	Moderate	0.033 globulin	Severe	0.1 whole egg white	Moderate
7..0.002 "	"	0.010 "	"	0.050 "	Doubtful	0.033 "	Moderate	0.1 " " "	Severe
8..0.001 "	Moderate	0.0025 "	Moderate	0.050 "	"	0.050 "	"		
9..0.001 "	"	0.0025 "	Doubtful	0.050 "	"	0.050 "	"		
10..0.001 "	Severe	0.0025 "	"	0.050 "	Slight	0.050 "	"		
11..0.001 "	"	0.0025 "	"	0.050 "	"	0.050 "	"		
12..0.050 ovomucoid A	Slight	0.050 ovomucoid A	"	0.050 "	Died in 25 min.				
13..0.050 " A	"	0.050 " A	"	0.050 "	" " 25 "				
14..0.050 " A	"	0.050 " A	"	0.050 "	" " 10 "				
15..0.010 " C	Moderate	0.040 " C	None	0.050 "	" " 30 "				
16..0.010 " C	"	0.040 " C	"	0.050 "	Severe	0.050 "	Severe		
17..0.010 " C	"	0.040 " C	"	0.050 "	"	0.050 "	None		
18..0.050 vitellin	Doubtful	0.003 albumin	Severe	0.050 "	None	0.050 "	Severe		
19..0.050 "	"	0.033 globulin	"	0.1 whole egg white	Severe				
20..0.050 "	"	0.033 "	Slight	0.1 " " "	"				

the refractory condition, but the amount of injected antigen. Of similar import is the well known phenomenon of polysensitization as demonstrated by Rosenau and Anderson, that a guinea-pig sensitized with several proteins is specifically sensitive to all, and will react with any one after recovering from anaphylactic shock induced by any one of the others.

Taking advantage of these features of the anaphylaxis reaction it is possible to exhaust a specific anaphylactin in the body of a guinea-pig, leaving the animal still sensitized to other proteins. In this way the different proteins of egg white have been studied as to their identity. Guinea-pigs were sensitized with entire egg white from fresh eggs (given in doses containing 0.006 gm. of protein), which, of course, contains all the different distinct proteins which there may be in egg white. Pigs sensitized in this way will react, after the usual incubation period, to any of our three fractions of egg white ("globulin," albumin, and ovomucoid) but only doubtfully to vitellin from the yolk. If the sensitized animal is given two or more injections of one of these fractions at 24 hour intervals, rendering it entirely refractory to this particular protein, it will be found to be still sensitive to either one of the other fractions or to the entire egg white. This demonstrates conclusively that there are several proteins or antigens present in the egg white, which are biologically distinct from one another, although derived from the same species.

By this means it can be shown that guinea-pigs sensitized to whole egg white and made refractory to "globulin" will still react strongly or fatally to crystallized albumin, or if made refractory to albumin they will still react to globulin. This proves that there are proteins or at least antigens present in each of these fractions which are not present in the other fraction. It also proves at the same time that even the extreme method of separation by fractional precipitation with ammonium sulphate which was used in making these preparations is not adequate to separate the proteins of egg white, since at least one common protein or antigen is present in each fraction. From the standpoint of chemistry nothing else could be expected, but it seems desirable to demonstrate and emphasize again the inefficiency of the salting-out method as a

means of separating proteins for biological study, in view of the persistent attempts which are made to do so. While the present work concerns only the proteins of egg white, there is no reason for believing that any more accurate fractionation can be obtained with serum or other natural mixtures of closely related proteins.

HYPERSENSITIZATION AND IMMUNITY BY FEEDING.

In a previous publication[1] evidence was cited which seemed to indicate that the feeding of a protein for a limited time renders a guinea-pig hypersensitive to the same protein, yet if the feeding was continued long enough the guinea-pig eventually becomes either immune or refractory. The experimental data at that time were limited, but further observations on this feature of anaphylaxis have given further support to the conclusions drawn.

The following observations have been made:

1. Guinea-pigs bred from mothers fed with oats were, as soon as weaned, put upon a diet of egg albumin and carrots. Other young pigs from the same stock were raised upon oats and carrots. The latter animals after reaching a weight of 250 to 300 gms. did not give anaphylactic reactions when injected with 0.05 gm. of a protein obtained from raw oats, and if given small doses, such as ordinarily given for sensitizing, they were not rendered sensitive to subsequent injections of 0.05 gm. of oat protein. Some of the pigs which were raised to 200 to 250 gms. weight without oats were found to give a typical reaction of moderate severity when injected once with 0.05 gm. oat protein, apparently from passive sensitization conferred by the mother. Others gave no reaction. After the animals fed without oats were somewhat older, 350 to 400 gms., they reacted much less strongly or not at all to oat protein, as if this inherited passive sensitization were passing off, as passive sensitization normally does; such pigs, if given sensitizing doses of oat protein, are found to be sensitive to this protein three weeks later, giving well defined reactions of moderate severity. Hence the conclusion seems warranted that if guinea-pigs are raised on oat proteins they cannot be made to give anaphylactic reactions with oat proteins, but if raised without oats they may be sensitized

[1] Wells and Osborne, *Jour. Infect. Dis.*, 1911, 8, p. 77.

to oat proteins, just as they can be to other proteins not usually in their food. These experiments support the experience obtained previously with zein, that guinea-pigs become immune to the chief vegetable proteins of their food.

2. Guinea-pigs raised from the time of weaning on a diet of egg protein (Merck's dried egg albumin) and carrots were found, as in previous experiments, to give strong anaphylaxis reactions when injected with egg albumin between the thirtieth and sixtieth days, but later they reacted less strongly, and after the one hundredth day of feeding they gave but slight reactions to 0.1 gm. dried egg albumin. At this time sensitization with egg albumin can be obtained to only a slight degree, such guinea-pigs given injections of egg albumin showing but slight reaction to a subsequent dose of egg albumin, while control pigs fed on oats and carrots gave severe, usually fatal, reactions to corresponding injections of egg albumin. Apparently, then, daily absorption of animal protein in the food at first renders guinea-pigs hypersensitive to this protein, but if the feeding is kept up for a long enough period the animals become refractory to the food protein.

3. A series of guinea-pigs which were raised on bread and cow milk for 10 weeks were found at the end of this time to be still highly sensitive to milk, dying promptly when given 1 to 3 c.c. of milk intraperitoneally. Apparently this length of feeding is not sufficient to render guinea-pigs immune to milk. These results are not at all in harmony with those of Besredka,[1] who did not succeed in sensitizing with milk by either oral or rectal administration, but did find that sensitized guinea-pigs were made refractory to intracerebral injections of milk if previously given milk by either of these routes.

DOES EGG WHITE CONTAIN A SUBSTANCE INHIBITING THE ANAPHYLAXIS REACTION?

In a set of experiments previously reported (I) it was found that the minimum amount of crude egg white required to produce sensitization or intoxication was much larger than would be expected from its known content of crystallizable egg albumin, to say

[1] *Ann. Inst. Pasteur.*, 1909, 23, p. 166.

nothing of the other proteins. Thus, in one experiment the minimum amount of protein in the form of crude egg white which sensitized guinea-pigs was one hundred times greater than the minimum sensitizing dose of crystallized egg albumin, and the minimum lethal dose was five times greater. As the crystallizable albumin constitutes over half of the total egg proteins, this difference in reaction cannot be ascribed to the fact that only part of the egg protein is albumin, and it was suggested that raw egg proteins might possibly contain some substance inhibiting the anaphylaxis reaction, at least to some extent.[1] A repetition of this experiment with other samples of crude egg white gave similar results, as shown in the following table:

COMPARATIVE ACTIVITY OF CRUDE AND PURE EGG PROTEINS.

Protein Used	Minimum Sensitizing Dose Causing Slight Reaction	Minimum Sensitizing Dose Causing Fatal Reaction	Minimum Fatal Intoxicating Dose
Crystallized albumin	0.000,000,05	0.000,001	0.000,5
Purified globulin	0.000,000,1	0.000,001	0.000,8
Crude egg white protein (I)	0.000,003	0.000,012	0.001
Crude egg white protein (II)	0.000,006	0.000,5	0.002,5

One possibility was that the different proteins interfere with one another in the reaction. To test this, experiments were performed in which animals were sensitized with one of the proteins of the egg white and then allowed to react with two different proteins; but in a long series of experiments no definite evidence of inhibition could be obtained in this way.

As it appeared from these experiments that the inhibition was not caused by any of the proteins, the effect of the non-protein material was then tried, with but little more positive results. It was found that when a few milligrams of the non-coagulable material, obtained by evaporating to dryness the filtrate from the coagulated whites of several eggs, were added to crystallized albumin, the amount necessary to produce fatal sensitization was apparently somewhat increased; but there was no definite interference with the toxicity of crystallized albumin or pure globulin for sensitized animals. In a few experiments minimum lethal

[1] Cabannes (*Compt. Rend. Soc. Biol.*, 1907, 62, p. 809) also states that the proteins precipitated by ammonium sulphate are more toxic than the totality of the protein of serum.

doses of albumin mixed with the residue failed to kill sensitized pigs, but in so many other cases this effect was not observed that the positive results cannot be considered as significant. This non-coagulable material, of course, includes ovomucoid, but as this protein was found not to affect the reaction, the slight results obtained must be ascribed to the non-protein constituents. Of these, the salts and the lipoids must be considered, and as yet we have not been able to investigate the responsibility of each of these groups of substances. Banzhaf and Steinhardt[1] found that egg lecithin affords no protection to an animal sensitized with serum if injected together with the second dose of serum, but if given intraperitoneally several hours previous to the second injection it protects quite effectively.

As the reactivity of egg globulin seems to be if anything helped rather than impaired by the non-coagulable residue, it may be thought that the salts are concerned, since small amounts of salt favor the solution of globulin, and also since it is known that large amounts of sodium chloride interfere with the union of amboceptor and complement, thus inhibiting the anaphylaxis reaction. But taken all together the results of my experiments are too inconclusive to locate any true inhibiting substance which may exist in the egg white. There still remains the possibility that a thermolabile substance is present in egg white and not present in either the purified albumin or globulin, but its existence has not been established by my experiments. Fein,[2] however, obtained results pointing to such a possibility, for he found that heating a serum to 58° C. may increase its anaphylactic toxicity, and in a way my results support his hypothesis.

EXPERIMENTS WITH CASEIN.

Purifed caseinogen was found to be highly efficient in producing anaphylactic reactions, giving more fatal reactions than did milk in corresponding doses, as is shown by Table 5. Thus, 0.1–0.25 gm. purified caseinogen of cow milk usually produced fatal reactions in sensitized guinea-pigs, while with other animals sensitized in the same way 5–10 c.c. of milk, containing 0.15–0.30 gm. of

[1] *Jour. Med. Res.*, 1910, 23, p. 6.

[2] *Centralbl. f. Bakt.*, 1909, 51, p. 576.

TABLE 5.
Casein.

	Sensitizing Dose	Days' Interval	Second Injection	Results	Subsequent Injections and Remarks
1....	0.025 gm. caseinogen, cow	21	0.25 gm. caseinogen, cow	Died in 15 min.	
2....	0.0025 " " "	21	0.25 " " "	" " 15 "	
3....	0.025 " " " (boiled)	17	0.16 " " "	" " 2 hrs.	The sensitizing caseinogen was heated 25 min. at 100°
4....	0.01 " " " "	17	0.16 " " "	" " 15 min.	" " " " " " " " "
5....	0.005 " " " "	17	0.16 " " "	Moderate symptoms	" " " " " " " " "
6....	0.01 " " "	21	10 c.c. cow's milk	" "	
7....	0.005 " " "	21	5 " " "	" "	
8....	0.025 " " "	17	10 " " "	Severe "	24 hrs. later no reaction to caseinogen
9....	5.0 c.c. cow's milk	17	0.16 gm. caseinogen, cow	Died in 35 min.	
10....	2.0 " " "	17	5.0 c.c. cow's milk	" after 2 hrs.	Died during night
11....	1.0 " " "	17	0.16 gm. caseinogen, cow	" in 2 hrs.	
12....	5.0 " " "	17	— goat casein	Doubtful symptoms	24 hrs. later reacted fatally to cow caseinogen
13....	0.01 gm. caseinogen, cow	26	— " "	Slight "	(Dosage with goat casein doubtful because of poor solubility)
14....	0.01 " " "	21	" "	" "	
15....	0.002 " " "	21	" "	Severe "	24 hrs. later reacted severely to cow caseinogen
16....	0.001 " " "	21	" "	No "	
17....	0.0002 " " "	21	" "	Moderate "	24 " " " " " " "
18....	0.1 " goat casein	21	0.1 gm. cow caseinogen	Died in 65 min.	
19....	0.002 " " "	21	0.1 " " "	" " 50 "	
20....	0.001 " " "	21	0.1 " " "	Moderate symptoms	24 " " " slightly " goat casein
21....	0.0002 " " "	21	0.1 " " "	" "	24 " " " " " " "
22....	0.01 " " "	26	0.1 " " "	Severe "	
23....	0.01 " " "	26	0.1 " " "	" "	

caseinogen, seldom produced fatal reactions. Heating of caseinogen to 100° for 25 minutes did not materially impair its efficiency in producing fatal anaphylactic sensitization (experiments 11, 12, 13), a result to be expected since it has already been shown,[1] and repeatedly confirmed, that heating of whole milk does not destroy its power of causing anaphylactic sensitization or intoxication.

A suggestive result is shown in experiments 16–24, which indicate that caseinogen from the goat and cow interact with one another. This agrees with Bordet's[2] observation that guinea-pigs sensitized to cow milk react with goat milk; also with Fleischer,[3] who studied precipitin reactions with purified casein from different species of animals, and obtained interreactions differing only in degree. Bauer[4] also found that by the means of the complement fixation reaction casein could be differentiated from lactalbumin of the same species much more readily than from casein of different but related species, the latter observation applying to cow and goat casein. Here we have a case in which chemically related proteins from different species show strong relationships by biological reactions, while naturally associated proteins in the same species, when of widely differing chemical nature, show very little relationship.

EXPERIMENTS WITH COMPOUND PROTEINS.

In view of the general interest in the biological reactions of the so-called nucleoproteins, a number of attempts were made to study the reactions of these proteins as well as of their components, but with practically no success. A large quantity of ripe cod testes was obtained from the Woods Hole station of the Bureau of Fisheries, through the courtesy of Superintendent E. F. Locke. From this was prepared a histone, the "Gadushistone," which has been studied by Kossel and Kutscher.[5] Also a sodium nucleinate was prepared, and from the sperm-free aqueous extract of the testicles a protein resembling albumin was separated by precipi-

[1] Wells, *Jour. Infect. Dis.*, 1908, 5, p. 449.

[2] *Ann. Inst. Pasteur*, 1909, 23, p. 166.

[3] *Roussky Wratsch*, 1908, 49; Abst. in *Centralbl. f. Pathol.*, 1909, 20, p. 308.

[4] "Ueber den Artcharakter der Milcheiweisskörper," *Berl. klin. Wchnschr.*, 1910, 47, p. 830.

[5] *Ztschr. Physiol. Chem.*, 1900, 31, p. 188.

tation with ammonium sulphate, and repurified by repeated precipitation.

The albumin behaved like ordinary serum albumin or egg albumin, producing typical and fatal anaphylactic reactions, and being specific when tried against mammalian sera.

The nucleinate did not produce any reactions when guinea-pigs were given small sensitizing and larger intoxicating doses (0.1 gm.) at a three weeks' interval; a result to be expected, since no protein is present in the preparation.

The histone was so toxic of itself that its anaphylactic properties could not be studied; guinea-pigs given 0.1 gm. of this preparation becoming severely ill in a few minutes and remaining so for hours, the symptoms being quite similar to those of anaphylactic shock. Normal animals were fully as much affected by this protein as were those which had been given sensitizing doses. The toxicity was apparently not at all reduced by heating the neutral solution for one-half hour at 56°.

These results are similar to those obtained by Taylor[1] with salmon sperm, which was found to be highly toxic to rabbits, while no cytolytic antibody was found by immunization of rabbits to the isolated protamine or to the nucleinic acid. McCrudden[2] also has found fish ovaries to contain very toxic substances causing death of rabbits in a few minutes after subcutaneous injections. This material, however, was found in the albumin fraction.

A preparation of nucleoprotein was made from dog livers according to the method described by Beebe. This preparation on hydrolysis yielded a large amount of purines, and resembled in all respects the compound proteins of this class. Injected into guinea-pigs in 0.1 gm. doses, dissolved in 0.1 per cent NaOH, it was found to be somewhat toxic, and normal guinea-pigs reacted if anything more strongly to the nucleoprotein than did previously injected animals. Heating to 56° for one-half hour did not greatly lessen the toxicity of this preparation. Guinea-pigs sensitized to this nucleoprotein preparation in 0.1 gm. doses gave moderate reactions with dog serum, but doses of one to two milligrams did not make guinea-pigs sensitive to dog serum. It seems probable

[1] *Jour. Biol. Chem.*, 1908, 5, p. 311. [2] *Ibid.*, 1911, 9, p. 9.

that the numerous chemical manipulations, especially the repeated solution in alkali and precipitation with acid, may be responsible for the inefficiency of these preparations of nucleoprotein and histone, for it is known that the action of acids and alkalies rapidly impairs the activity of proteins in respect to anaphylaxis, as well as other biological reactions.[1]

Interest in compound proteins having been aroused by the nucleoprotein work, experiments were made with another sort of compound protein, a *mucin* prepared from the gastric mucosa of pigs, and kindly furnished me by Professor L. B. Mendel. This glycoprotein was found capable of producing anaphylactic intoxication in sensitized guinea-pigs, the reactions generally being of moderate severity, but one fatal reaction occurring in six experiments. Animals sensitized with pig serum or with an extract of pig muscle tissue did not react to mucin, and the mucin did not render them refractory to pig serum. Some slight reactions were obtained when muscle extract was injected into the guinea-pigs sensitized with the mucin preparation. As this reaction occurred only in this direction it is probably to be interpreted that some traces of muscle or blood proteins were present in the mucin preparation. In any event, we have here another case of a protein from an animal showing a well developed independent biological specificity against the serum and tissues of the same species of animals.

ACTION OF AUTOLYTIC AND TRYPTIC ENZYMES UPON ANAPHYLACTIC ANTIGENS.

Previous experiments having shown that digestive enzymes destroy the anaphylactic properties of proteins without altering their specificity, a few experiments were performed to test the influence of autolysis in this respect. A lot of human placentas were allowed to autolyze two years under toluene in five volumes of water, at room temperature. The toluene was removed from a portion of the supernatant fluid, which contained 10 mg. of coagulable protein per c.c., and this protein solution was used. It was found to cause typical reactions, often fatal, when injected into guinea-pigs sensitized with either human serum or with the autoly-

[1] See *Jour. Infect. Dis.*, 1909, 6, pp. 509–13.

sis fluid. Guinea-pigs sensitized with the autolysis fluid reacted severely or fatally to human serum, but not at all to dog serum or horse serum. Apparently, then, this prolonged autolysis, not having removed all the coagulable protein, has not destroyed the sensitizing or intoxicating properties of the proteins in the solution; neither has it destroyed their specificity.

In this connection may be mentioned a continuation of an experiment on the destruction of the sensitizing property of serum by trypsin. It was previously reported (article II) that a specimen of bovine serum which had been digested with commercial pancreatic powder (pig pancreas) for 314 days at 37°, with fresh pancreatic powder added at intervals, still retained the power of sensitizing guinea-pigs to bovine serum when doses of 1 c.c. or more were used, but no anaphylactic intoxication could be demonstrated with 10 c.c. doses of the digestion mixture injected into sensitized guinea-pigs. The digestion of this same preparation of bovine serum has been continued, a small amount of pancreatin being added every few months. At the end of the second year of digestion the smallest amount of digestion mixture sensitizing an animal was 6 c.c., and at the end of three years only a slight reaction could be obtained when 10 c.c. sensitizing doses were used, but none at all with 5 and 7.5 c.c. sensitizing doses. Therefore three years' digestion has not entirely extinguished the power of this serum to sensitize guinea-pigs, although the degree of activity is extremely slight, when it is considered that fresh serum sensitizes in doses of 0.0001 c.c. to 0.00001 c.c.

SUMMARY.

Ovovitellin from the yolk of hen eggs when tested by the anaphylaxis reaction was found to be entirely distinct from crystallized egg albumin, and from ovomucoid from hen egg white, and also from vitellin from the yolk of turtle eggs.

Ovomucoid produces characteristic and specific anaphylaxis reactions, in spite of protracted boiling and repeated precipitation with alcohol. It is entirely distinct, according to this reaction, from crystallized egg albumin, from repurified "globulin" of egg white, and from ovovitellin, all from hen eggs.

The "globulin" fraction of hen egg white has about the same degree of intoxicating and sensitizing power as crystallized egg albumin; if anything it is slightly less active. In spite of the most careful separation of these two portions of egg white by means of ammonium sulphate precipitation, the resulting preparations each react almost as well against the other as against itself.

Guinea-pigs sensitized with several antigens can be saturated with one of these antigens and then will react severely to one of the others. By this means it can be shown that there are in the "globulin" fraction and in the crystallized albumin, specific and distinct antigens, and also a common antigen which cannot be separated from them by fractional precipitation with ammonium sulphate.

Therefore it is demonstrated that in hen egg white there are at least four antigens (three in the coagulable protein and one non-coagulable) which can be distinguished by the anaphylaxis reaction, and therefore are biologically distinct from one another although coming from a single secretion. At least one other protein of the egg, the ovovitellin, can be differentiated from the other three egg antigens, so that we have here demonstrated five antigens in the egg which are biologically distinct in spite of a common origin. These must be looked upon as examples of chemical specificity independent of species specificity. The antigens distinguished by the anaphylaxis reaction seem to correspond to the proteins which have been distinguished by chemical means.

Guinea-pigs fed upon a certain protein are at first rendered sensitive to this protein. After some time, however, if the feeding is continued they become less sensitive, until they reach an immune or refractory condition so that they do not react to two spaced injections of the fed protein. This refractory condition seems to be reached more easily with the vegetable proteins of the natural food (corn, oats) than with animal proteins.

Crude egg white has less sensitizing and intoxicating power than corresponds to the activity of the isolated proteins from equal amounts of egg white. It has not been possible to determine the nature of the inhibiting substance which accounts for this depression of the activity of the proteins of crude egg white.

Pure caseinogen gives strong anaphylaxis reactions, and the casein of cow milk and of goat milk react freely against each other.

Sodium nucleinate from cod sperm does not give anaphylaxis reactions. Albumin from cod sperm gives typical and specific anaphylaxis reactions. Histone from cod sperm is toxic to guinea-pigs, retaining its toxicity when heated to 56°, and not producing demonstrable anaphylaxis reactions.

Mucin from the pig's stomach produces typical anaphylaxis reactions, and is specific against pig's blood serum or extracts of pig muscle.

Autolysis of two years' duration, but without destruction of all coagulable proteins, did not destroy the anaphylactic antigen of human placenta extract, and did not destroy its specificity. Three years' digestion of bovine serum with trypsin almost but not quite destroyed its sensitizing power, the minimum sensitizing dose being 10 c.c.; at the end of two years' digestion good reactions were obtained when the sensitizing dose was 6 c.c.

THE BACTERICIDAL ACTION OF QUINONE AND OTHER PHENOL OXIDATION PRODUCTS AS DETERMINED BY THE RIDEAL-WALKER METHOD.*

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The method used in this work was the Rideal-Walker method for standardization of disinfectants.

The dilutions of the disinfectants to be tested were placed in ordinary wide mouthed test-tubes in five c.c. amounts and tested in series of 10 tubes each. One-half of a cubic centimeter of a 24 hour broth culture of *B. typhosus* was added to each of these test-tubes at 15 second intervals.[1] Six subinoculations of each of these were made into tubes containing 10 c.c. of broth, at intervals of $2\frac{1}{2}$ minutes. The process of adding the culture to the mixing tubes containing the disinfectant occupied $2\frac{1}{4}$ minutes, allowing 15 seconds before it is necessary to make a subculture from tube number one. Proceeding then with subcultures at 15 second intervals, the time factor takes care of itself and we have a series of six subcultures from each mixing tube at intervals of $2\frac{1}{2}$ minutes, i.e., from a $2\frac{1}{2}$ minute exposure to the action of the disinfectant on the culture to one of 15 minutes. The tubes of broth were kept in racks to facilitate inoculation, and an assistant held up the tube to be inoculated. This assistant, however, is not necessary. The 15 second interval allowed was found to be ample, during which time the loop had to be flamed and allowed to cool. The inoculated tubes were then incubated at 37° C. for 48 hours, then read and tabulated. Plus (+) indicates growth, minus (−) no growth.

The material used.—All the glassware used was previously sterilized, as was also the distilled water for making the various dilutions of the disinfectants. The original dilutions were made by accurately weighing out the material on a chemical balance and

* Received for publication June 19, 1911.

[1] A definite measured amount of culture was added in preference to the drop method of Rideal and Walker.

diluting this in a standardized graduated flask, and the subsequent dilutions were made from this with standardized graduated pipettes. The loop used for subinoculations was two mm. in diameter and made of very fine platinum wire so that it would cool rapidly. A full loopful was transferred in every case.

The phenol coefficient was determined according to the Rideal-Walker method, namely: "by dividing the figure indicating the degree of dilution of the disinfectant that kills an organism in a given time by that expressing the degree of dilution of carbolic acid that kills the same organism in the same time under exactly similar conditions."[1]

There are two important, improved modifications of this method, as follows:

"The important modifications of the Lancet method on the Rideal-Walker are, in the increased number of dilutions employed, sometimes as many as 12 tubes being inoculated in each $2\frac{1}{2}$ minute interval; extension of the number of time intervals to 30 instead of 15 minutes; the use of *B. coli* and MacConkey's bile salt media for subcultures instead of *B. typhosus* and standard extract broth; the amount of the mixture of culture and disinfectant transferred to the subculture tubes; the method of determining the coefficient.

"The coefficient, as determined by the Lancet Commission, is arrived at as follows: The figure representing the percentage strength of the weakest killing dilution of the phenol is divided by the figure representing the percentage strength of the weakest killing dilution of the unknown disinfectant, both at $2\frac{1}{2}$ and at 30 minutes, the mean resulting figure being taken as the true coefficient. An example of the determination of the carbolic acid coefficient by the Lancet method may be seen from the following table:"

[1] Taken from paper of Anderson and McClintic.

DISINFECTANT X.

Minutes	Dilutions							
	1–300	1–400	1–500	1–600	1–700	1–800	1–900	1–1,000
	Per cent 0.333	Per cent 0.250	Per cent 0.200	Per cent 0.166	Per cent 0.143	Per cent 0.125	Per cent 0.111	Per cent 0.100
$2\frac{1}{2}$	o	o	+	+	+			
5		o	o	+	+	+		
$7\frac{1}{2}$		o	o	+	+	+		
10			o	+	+	+	+	
$12\frac{1}{2}$			o	+	+	+	+	
15				+	+	+	+	+
20				o	+	+	+	+
25				o	+	+	+	+
30				o	+	+	+	+

CARBOLIC ACID CONTROL.

Minutes	Percentage Dilutions							
	1.10	1.00	0.917	0.846	0.786	0.733	0.687	0.647
$2\frac{1}{2}$	o	o	+	+				
5	o	o	+	+				
25					o	o	+	+
30					o	o	+	+

Room temperature 67° F.; + signifies growth; o signifies no growth; blank spaces signify not tested.

The coefficient is therefore:

$$\frac{\frac{1.00}{0.25}+\frac{0.733}{0.166}}{2}=\frac{4.0+4.4}{2}=4.2$$

The Hygienic Laboratory method uses a measured amount of 24-hour broth culture of *B. typhosus*, constant temperature in water bath for mixing tubes, a loop four mm. in diameter for making subinoculations, and the following modification of the Lancet method of determining the coefficient:

"After a large number of experiments we have concluded that the method employed by the Lancet Commission, with certain modifications, is the best one for determining the coefficient, i.e., the mean between the strength and time coefficients.

"In performing the test, plants are made every $2\frac{1}{2}$ minutes up to and including 15 minutes. The method of determining the coefficient will be seen in table."[1]

[1] Taken from paper of Anderson and McClintic.

Date, May 18, 1910.

Name "A."
Temperature of Medication, 20° C.
Culture Used, *B. typhosus*, 24 hr., Extract Broth, Filtered.
Proportion of Culture and Disinfectant, 0.1 cc.+5 c.c.
Organic Matter, None. Kind, None, Amount, None.
Subculture Media, Standard Extract Broth. Reaction, 1.5. Quantity in Each Tube, 10 c.c.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:						Phenol Coefficient
		2½	5	7½	10	12½	15	
Phenol	1:80	−	−	−	+	+	+	
	1:90	+	−	−	−	+	+	80)375 4.69
	1:100	+	+	+	−	−	−	
	1:110	+	+	+	+	+	−	110)650
								5.01
Disinfectant "A"	1:350	−	−	−	+	+	+	2)10.60
	1:375	−	−	−	+	+	+	5.30=
	1:400	+	−	−	−	+	+	Coefficient
	1:400	+	−	−	−	+	+	
	1:425	+	+	−	−	−	−	
	1:450	+	+	−	−	−	−	
	1:500	+	+	−	−	−	−	
	1:550	+	+	+	−	−	−	
	1:600	+	+	+	+	−	−	
	1:650	+	+	+	+	+	−	
	1:700	+	+	+	+	+	+	
	1:750	+	+	+	+	+	+	

While making a series of tests for the standardization of some coal tar disinfectants, two samples of phenol were used; one had been in the laboratory a long time and had become slightly pink and the second was a recently purchased, colorless preparation. It was observed in a number of tests (see Table 1) that the colored phenol consistently showed a more powerful bactericidal action than the colorless phenol.

TABLE 1.

Date, February 12, 1911.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:					
		2½	5	7½	10	12½	15
Phenol "N" (colorless)	1–70	−	−	−	−	−	−
	1–80	−	−	−	−	−	−
	1–90	+	+	+	+	−	−
	1–100	+	+	+	+	+	+
Phenol "O" (light pink color)	1–70	−	−	−	−	−	−
	1–80	−	−	−	−	−	−
	1–90	+	−	−	−	−	−
	1–100	+	+	+	−	−	−
	1–110	+	+	+	+	+	−
	1–120	+	+	+	+	+	+

This suggested that, in this process of coloring, products of a stronger bactericidal action were formed, and that perhaps we would find this action increased with the increase in color in the phenol, and that these substances could be isolated and investigated separately.

H. D. Gibbs has shown that this process of coloring is an oxidation process and occurs in the presence of oxygen when phenol is exposed to sunlight and also slowly in the dark, but not when in contact with indifferent gases, such as hydrogen, nitrogen, and carbon dioxide. Also, that active oxygen will unite with phenol with considerable ease and rapidity, and ozone is very reactive. The principal substances formed are quinone, quinol, and catechol, and the principal colored compounds are probably quinone condensation products. The intense red product is probably phenol-quinone.

We then secured three samples of colored phenol of different intensity and submitted them to the test. Sample "K" was a bright cherry red, phenol "N" was the colorless specimen, phenol "O" the slightly colored specimen originally tested, phenol "J" was intermediate between "O" and "K."

As Table 2 shows, there is an increase in the bactericidal action through phenols "O" and "J," but "K," which is the most colored, is but slightly stronger than "N," which is colorless.

TABLE 2.

Date, April 8, 1911.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:					
		2½	5	7½	10	12½	15
Phenol "N" (colorless).......	1–70	–	–	–	–	–	–
	1–90	+	+	+	+	–	–
	1–100	+	+	+	+	+	–
	1–110	+	+	+	+	+	+
Phenol "O" (light pink color)	1–70	–	–	–	–	–	–
	1–90	+	–	–	–	–	–
	1–100	+	+	+	+	+	+
	1–120	+	+	+	+	+	+
Phenol "J" (color intermediate between "O" and "K")	1–90	–	–	–	–	–	–
	1–100	–	–	–	–	–	–
	1–110	+	+	–	–	–	–
	1–130	+	+	+	+	+	+
Phenol "K" (cherry red color)	1–70	–	–	–	–	–	–
	1–90	+	+	–	–	–	–
	1–100	+	+	+	+	+	+

As this may have been due to "K" being an inferior product and deficient in phenol, we next exposed to sunlight for different periods of time three samples of the colorless phenol "N," and obtained specimens of increasing degree of coloration. These were tested and the results shown in Table 3 were disappointing as they show no consistent increase of bactericidal action with increase in density of color of phenol or time of exposure. The only explanation we can offer is that the marked color present, due to these highly colored benzene compounds, must be caused by quantities insufficient to affect the test. There are, however, no chemical methods capable of testing this point.

TABLE 3.

Date, May 7, 1911.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:					
		2½	5	7½	10	12½	15
Phenol "N" (desiccated)	1–90	−	−	−	−	−	−
	1–100	+	+	−	−	−	−
	1–110	+	+	+	−	−	−
	1–120	+	+	+	+	+	−
	1–130	+	+	+	+	+	+
Phenol "N" (unexposed)	1–80	−	−	−	−	−	−
	1–90	+	−	−	−	−	−
	1–100	+	−	−	−	−	−
	1–110	+	+	+	+	−	−
	1–120	+	+	+	+	+	+
Phenol "N" (exposed April 14–18)	1–80	−	−	−	−	−	−
	1–90	+	+	−	−	−	−
	1–100	+	+	−	−	−	−
	1–110	+	+	+	+	+	−
	1–120	+	+	+	+	+	+
Phenol "N" (exposed April 14–26)	1–80	−	−	−	−	−	−
	1–90	+	−	−	−	−	−
	1–100	+	−	−	−	−	−
	1–110	+	+	−	−	−	−
	1–120	+	+	+	+	+	+
Phenol "N" (exposed April 14–May 7)	1–80	−	−	−	−	−	−
	1–90	+	−	−	−	−	−
	1–100	+	+	−	−	−	−
	1–110	+	+	+	+	−	−
	1–120	+	+	+	+	+	+

While waiting for the above sample to become colored we tested quinone and phenol-quinone, which were the only phenol oxidation products available to us, and also tested hydroquinone because of its close relationship to quinone as a reduction product. In view of the above these results were rather startling and are recorded in Table 4.

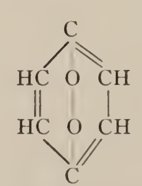
TABLE 4.

Date, April 11, 1911.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:						Phenol Coefficient
		$2\frac{1}{2}$	5	$7\frac{1}{2}$	10	$12\frac{1}{2}$	15	
Quinone	1–600	−	−	−	−	−	−	100)16,000
	1–1,000	+	−	−	−	−	−	160 = Coefficient
	1–1,500	+	−	−	−	−	−	(Rideal-Walker)
	1–2,000	+	−	−	−	−	−	
	1–2,500	+	+	−	−	−	−	70)600 8.6
	1–3,000	+	+	+	−	−	−	100)16,000 160.0
	1–4,000	+	+	+	−	−	−	2)168.6
	1–4,500	+	+	+	−	−	−	84.3 =
	1–5,000	+	+	+	−	−	−	Coefficient
	1–10,000	+	+	+	−	−	−	(Hygienic Lab.)
	1–16,000	+	+	+	+	+	−	
Hydroquinone	1–100	+	+	+	−	−	−	80)200
	1–200	+	+	+	−	−	−	2.5 = Coefficient
	1–400	+	+	+	+	+	+	(Rideal-Walker)
Phenol-Quinone (freshly made and fresh solution)	1–800	−	−	−	−	−	−	70)800 11.4
	1–1,000	+	−	−	−	−	−	100)6,000* 60.0
	1–1,500	+	+	−	−	−	−	2)71.4
	1–2,000	+	+	−	−	−	−	35.7 =
	1–4,000	+	+	+	+	−	−	Coefficient
	1–8,000	+	+	+	+	+	+	(Hygienic Lab.)
Phenol "N" (colorless)	1–70	−	−	−	−	−	−	90)4,000
	1–90	+	+	+	+	−	−	44 = Coefficient
	1–100	+	+	+	+	+	−	(Rideal-Walker)
	1–110	+	+	+	+	+	+	

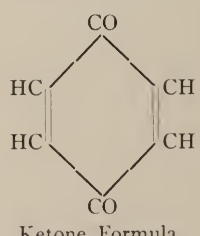
* Deduced from the table.

This table shows that the Rideal-Walker phenol coefficient of quinone is 160, which, as far as we have been able to discover, is the highest reported for any substance. Its formula is



Peroxide Formula
(1867, Graebe, *Z. f. Ch.*, N.F. 3, 39.)

or



Ketone Formula
(Fittig, A. 180, 23.)

and it is a very powerful oxidizing agent, its oxygen atoms being especially loosely bound, and its bactericidal action is undoubtedly to be attributed to this and not to its phenol moiety, for hydroquinone, with the two oxygen atoms replaced by hydroxyl groups, has a phenol coefficient of only 2.5.

It is also interesting to note that the activity of quinone is reduced when it is joined with the less active phenol to form phenol-quinone (coefficient 44). This latter is an unstable compound in solution and breaks down readily on standing, forming a muddy fluid. Quinone, which has a penetrating, irritating odor, is more stable in solution.

Our experience with the Rideal-Walker method has demonstrated to us that it has the defects pointed out by Anderson and McClintic of the Hygienic Laboratory and by the Lancet Commission. The most serious of these, as is shown by the above tables, is the variation of the bactericidal action directly with the increase in temperature. These tests were made at room temperature and very definitely demonstrate an increased bactericidal action with the approach of warm weather. The comparison of the activity of the disinfectant has consequently always been made with that of the standard phenol similarly tested at the same time under exactly similar conditions. Therefore the phenol coefficients determined at different times are comparable one with the other. In the choice of the dilutions for calculating the phenol coefficient, too much variation is allowed by the choice and personal equation of the investigator.

While the tables show some irregularity, we do not believe it sufficient to vitiate the results, especially as we have been able to closely confirm them by a number of repetitions of the work. Whereas one probably cannot exactly fix a phenol coefficient with the above method one can certainly determine the comparative germicidal efficiency of a series of disinfectants.

From the above it seems clear that very little, if any, increase in the bactericidal action occurred in the phenol exposed to the sun and also that quinone and phenol-quinone are many times more active than phenol; quinone being the most active substance known.

Our work was begun with the Rideal-Walker method and completed with the same for the sake of consistency of results. The Hygienic Laboratory phenol coefficient method we believe is a distinct advance over that of the Lancet Commission and we intend using it in any future work.

In the articles by Anderson and McClintic and the Lancet Commission there is a complete review of the progress of study of the standardization of disinfectants.

In conclusion we wish to take this opportunity of expressing our gratitude to Dr. J. H. Kastle for his kind interest and our indebtedness for his many suggestions which have guided us in this work. We wish also to express our indebtedness to Mr. Etheridge, of the Second Year Medical Class, for his assistance in the tests.

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A COMPARISON OF THE BACTERICIDAL ACTION OF QUINONE WITH THAT OF SOME OF THE COMMONER DISINFECTANTS.*

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This work was undertaken to furnish a basis of comparison of the bactericidal action of quinone and other phenol-oxidation products with some of the commoner disinfectants.

It is only in comparatively recent years, since the Rideal-Walker method for standardizing disinfectants was brought forward in 1903, that there has been in existence a bacteriological method which in the hands of different workers has given consistent results allowing of comparison. The principle of this method has been elaborated and further refined by the Lancet Commission and Anderson and McClintic of the Hygienic Laboratory, Washington, so that the latter method may be considered as being one of scientific exactness.

With these trustworthy means of investigation at our disposal, new and startling facts have been brought forward as regards both the efficacy and the inefficacy of many substances in common use as antiseptics and disinfectants. The Hygienic Laboratory workers from their studies of various proprietary disinfectants on the market have come to the conclusion of the fraudulent nature of many of them, and have undertaken an exhaustive investigation of these materials. The surgical profession has begun to discard some of its old antiseptic stand-bys, because of practical and common-sense reasons, even before modern research has shown the advantage that other substances and a simpler technic have over those formerly used. The surgeons are, of course, far removed from the days of "anti"-sepsis and the carbolic spray. The recognition of the impossibility of sterilizing the deeper layers of the skin has relieved most of them of the necessity of chlorinating the skin, etc. They

* Received for publication June 19, 1911.

are slow, however, in getting away from potassium permanganate and oxalic acid, and especially from bichloride of mercury.

The previous standardization of the bactericidal action of various substances done by Sternberg, Welch, and others will all have to be revised by our new methods and many steps in this direction have already been taken, to some of which we wish to call attention and compare with our own results. It must be realized that the figures give merely a comparison, under the special conditions of the test, of the bactericidal action of the various substances with phenol, but we are still left in the dark as to the efficiency of these materials under practical conditions. This very seeming defect makes the result more reliable as a bactericidal index, inasmuch as these special conditions admit of being made fixed and the same by different workers in different laboratories, thereby allowing a direct comparison of their results. These figures are, therefore, undoubtedly a rough indication of the practical value of the disinfectants, and as such, an indication for their practical use. Much work still remains to be done in the investigation of the practical side of this problem and many factors will have to be studied, such as the nature of the solvent or emulsion, the effect of the presence of organic matter, the effect of temperature, the relation of disinfection to the time element and to the nature of the bacteria to be destroyed, etc. Some of these points are already on a scientific basis. Thus, Lister pointed out that a solution of phenol in oil has little or no antiseptic action and this observation was confirmed by Koch. Chick and Martin have shown that the minute particles of a satisfactory emulsion exhibit a Brownian motion, are densest in the vicinity of the comparatively large bacteria, actively bombard them and are adsorbed by them, and that when free access to the bacteria is possible, substances show a greater bactericidal action when in the state of an emulsion than in aqueous solution. Other organic materials show similar and equal adsorptive actions, and so reduce by their presence the bactericidal action of this type of preparation, while aqueous solutions that do not coagulate organic matter are better able to penetrate through it to the bacteria and destroy them. Whatever the characteristics of these products, their essential basis must be an efficient bactericide.

These same observers have shown "that the reaction velocity of disinfection increases with the rise of temperature in a manner similar to that of chemical reaction." The importance of the time element in the disinfection process is also recognized and put by some on a similar chemical basis, this factor being taken advantage of in the recent methods of standardization. The difference in resistance of various bacteria, from the standpoint of obtaining results which will admit of comparison, will be an especially difficult problem to investigate.

These problems, however, belong to the future, and, with the general activity everywhere present in public health work, we trust that their investigation will be in the near future. They belong to the large, well equipped, resourceful municipal laboratories of our large cities.

In the review of recent work on standardization of disinfectants the report of the Lancet Commission[1] stands out as a stimulus to other workers and as an exhaustive

[1] See the original articles for description of the Rideal-Walker, Lancet, and Hygienic Laboratory methods of determining the phenol coefficient of a disinfectant.

work on the theory of action of bactericidal substances and the chemical and bacteriological nature of their composition and action. This report is an investigation of nineteen proprietary coal tar disinfectants marketed in England. It divides these disinfectants into two groups, those which form emulsions with water and those forming clear solutions. The first group comprises 14 substances and is subdivided into two classes, those with high and those with low phenol coefficients. There is a sharp difference between these, the high varying between 9.8 and 6.4 and the low between 2.2 and 0.75. The report states that no coefficient was obtained higher than 13. The second group includes five substances and these disinfectants forming clear watery solutions have, under the conditions of the test, relatively low coefficients compared with those of the emulsifying group. Crude carbolic acid (which did not form a clear solution for us) leads with a coefficient of 4.2, trikresol gives 2.5, and lysol 1.7. These results bring out in a striking way the relatively marked bactericidal action of the emulsifying coal tar disinfectants as compared with those forming a clear solution in water. In light of this we wish to note that in some of our tests with similar emulsions we have several times observed more activity in a 1–200 dilution than in a dilution of 1–100, a result we have attributed to increased penetrating power due to the more perfect emulsion at the higher figure. Of course this does not continue farther but shades off when the dilutions become excessive.

Recently some very interesting work has been reported by Post and Nicoll, which, however, greatly loses in its value because they have not used any of the standard methods. Their method, which is a modification of that of Rideal-Walker, is as follows: 0.5 c.c. of disinfectant and one loopful of a broth suspension, made from blood agar tube of the organism to be tested, are brought together; and inoculations of blood agar tubes, from which plates are poured, are made at 1, 10, and 30 minutes and 20 hours. These are incubated and the number of colonies are then counted and compared. They used four organisms: typhoid, pneumococcus, gonococcus, and streptococcus. No phenol coefficient was devised.

Blood agar is certainly an unnecessary refinement when working with the typhoid bacillus, which is the only organism which should be considered, as the other three are too variable in their different strains to be used by different workers. The small amounts of materials used must permit large percentage errors in the results. In spite of these and other defects in the method the uniformity and consistency of the results speak for themselves.

They come to the following conclusions:

1. "The reliability of the prompt action of a few simple germicides such as tincture of green soap, alcohol in dilutions of 50 per cent or over, silver nitrate solution as dilute as 1–1,000, iodine solutions, either as tincture or in aqueous solutions along with potassium iodide, and phenol in 5 per cent solution.

2. "The unreliability of many agents prevalently supposed to be effective germicides.

3. "The slow action of mercuric chloride, although when given hours to act it is effective in great dilutions."

Their tables must be consulted to appreciate the above.

Of the inefficient substances the slow action of bichloride of mercury stands out as the most important practical result. It would seem that this must soon be appreciated by the surgical profession, and that this substance will pass into retirement with

its predecessor, the carbolic spray. Already great advances have been made toward a simplified technic with proved, efficient antiseptics. Formaldehyde or rather formalin was shown not to kill the typhoid bacilli in 30 minutes in 1 per cent solution. This result we have been able to confirm. Argyrol 50 per cent and protargol 10 per cent are much less efficient than silver nitrate, 1–1,000.

Among the substances which stand out as sufficiently efficient to warrant attention and also wider adoption of their use are iodine, 50 per cent alcohol, silver nitrate, and tincture of green soap. They found complete killing of *B. typhosus* in one minute by tincture of iodine, by 1–400 solution of iodine in potassium iodide and water, tincture of green soap, and 50 per cent alcohol and great efficiency of 1–1,000 silver nitrate. The action of the tincture of green soap, as the authors suggest, is undoubtedly due to the alcohol present. We have been able to follow the aqueous solution of iodine in high dilutions with similar positive results. This latter substance is being used more and more in practice, such as application on the skin to prepare fields for operation, in solution for irrigation of infected wounds, the tincture painted on sluggish ulcers, for preparation of sterile iodine catgut, etc. The valuable work of A. V. Moschcowitz on the experimental and practical investigation of iodine catgut has shown this to be a most satisfactory preparation and has resulted in an extremely simple method for its preparation.

Seelig and Gould have recently reported some very interesting results on "Osmosis as an Important Factor in the Action of Antiseptics," and here again show the efficiency of iodine. Working with typhoid cultures contained in celloidin capsules, pouches of living rabbit mesentery, omentum, and skin, and excised rabbit diaphragm, and surrounded by various antiseptics, it was found that alcohol was most efficient as a bactericide, because of its combined bactericidal action and its rapid ability to osmose, which later property varied directly as its strength (up to 95 per cent). Tincture iodine acted even more quickly than plain alcohol. Mercury bichloride acted very poorly, showing a low grade of osmosis. Iodine penetrated the entire thickness of skin in one hour, but did not kill the typhoid bacilli. Rubbing skin with castor oil aided penetration of alcohol, and was used as proof that oil in the skin helps the penetration of alcohol and iodine and for this reason should not be removed by washing before this method of preparing a surgical field. This penetrating power of iodine seems to us most important.

Another ingenious method of testing the bactericidal and penetrating powers of disinfectants is that of Kendall and Edwards, which is briefly as follows: A mixture of definite quantities of a 24 hour broth culture of *B. coli* and sterile 1.5 per cent agar medium is allowed to harden in a sterile tubular mold 1.5 cm. in diameter. Pieces are then cut with a sterile knife at 2 cm. intervals and dropped into a beaker containing the disinfectant solution. These are removed at hour intervals with a special wire gauze forceps, washed with sterile water, and a core 3 mm. in diameter is removed in turn from their center and placed in No. 1 lactose broth fermentation tubes. These are incubated and the results as to growth after several days read and tabulated. A 5 per cent solution of phenol is used for comparison and a phenol coefficient is determined. The following table copied from their article is the report of all the substances they have tested. The relative efficiency of formalin is to be noted but the long time element does not indicate it to be very active. Bichloride acted only in strong solutions.

TABLE SHOWING COMBINED GERMIDICAL AND PENETRATING POWERS OF SOME COMMON DISINFECTANTS (KENDALL AND EDWARDS).

Agar 1.5 per cent. 72 hour incubation at body temperature. Temperature of exposure 20 degrees.

Disinfectants	Dilution	Time of Exposure		
		1 hour	3 hours	5 hours
Carbolic acid	5 per cent	+	+	—
Carbolic acid	1 " "	+	+	+
Formalin	4 " "	—	—	—
Formalin	1 " "	+	—	—
Formalin	0.25 " "	+	+	—
$HgCl_2$	0.1 " "	+	+	+
$HgCl_2$	1 " "	+	—	—
Chloride of lime	10 " "	+	+	—
Chloride of lime	4 " "	+	+	+
Hyco	2 " "	+	+	+
Cresol	1 " "	+	+	+
Sulphonaphthol	2 " "	+	+	+

\+ equals growth.
— equals no growth.

Further evidence of the bactericidal activity of mercuric chloride is given by Reymann and Nyman, who, using the method of Krönig and Paul, found its action less than that of silver nitrate.

H. C. Wood in a recent résumé of the subject of intestinal antiseptics mentions beta-naphthol as the substance best adapted for this use. He states that it is bactericidal at a dilution of 1–10,000, but does not mention the nature of the test for determining this figure, or the time it takes for this action to occur. We refer the reader to our data on this subject.

Our results with a few substances, using the Rideal-Walker method as outlined in a previous article, we submit in the following tabulated form.

TABLE 1. APRIL 23, 1911.
ORGANISM: *B. typhosus*.

Sample	Dilution	Time of Culture Exposed to Action of Disinfectant in Minutes:						Rideal-Walker Phenol Coefficient
		2½	5	7½	10	12½	15	
Hydrocyanic acid	1–100	+	+	+	+	—	—	110)100
	1–100	+	+	+	+	+	+	.9
Potassium cyanide	1–100	+	+	+	+	+	+	Coefficient not determined but less than 1
Formalin { Sat. sol. of formaldehyde in water	1–100	+	+	+	+	+	+	(Ditto)
Trikresol	1–200	—	—	—	—	—	—	90)200
	1–200	+	+	+	+	—	—	2.2
Parakresol	1–100	—	—	—	—	—	—	70)200
	1–200	+	—	—	—	—	—	2.8
Phenol	1–70	+	—	—	—	—	—	
	1–90	+	+	+	+	—	—	
	1–100	+	+	+	+	+	—	
	1–110	+	+	+	+	+	+	

\+ equals growth.
— equals no growth.

TABLE 2. MAY 4, 1911.

Organism: *B. typhosus.*

Sample	Dilution	Time of Culture Exposed to Action of Disinfectant in Minutes:						Rideal-Walker Phenol Coefficient
		2½	5	7½	10	12½	15	
Crude phenol	1–300	–	–	–	–	–	–	100)400 / 4
	1–400	+	–	–	–	–	–	
Iodine and potassium iodide	1–100	–	–	–	–	–	–	80)8,000 / 100
	1–8,000	–	–	–	–	–	–	
	1–10,000	+	+	+	+	+	+	
Sodium hydroxide	$\frac{n}{10}$ = 1–250	–	–	–	–	–	–	110)2,500 / 23
	$\frac{n}{20}$ = 1–500	–	–	–	–	–	–	
	$\frac{n}{50}$ = 1–1,250	+	–	–	–	–	–	
	$\frac{n}{100}$ = 1–2,500	+	+	+	+	–	–	
Ethyl alcohol	95 per cent	–	–	–	–	–	–	
	25 per cent	+	+	+	+	+	+	
Beta-naphthol in NaOH, $\frac{N}{10}$	1–100	–	–	–	–	–	–	100)200 / 2
Beta-naphthol in NaOH, $\frac{N}{20}$	1–200	+	–	–	–	–	–	
Beta-naphthol in NaOH, $\frac{N}{50}$	1–500	+	+	+	+	+	+	
Beta-napthol in 25 per cent alcohol	1–1,000	–	–	–	–	–	–	80)1,000 / 12.5
	1–2,000	+	+	+	+	+	+	
Phenol	1–80	–	–	–	–	–	–	
	1–90	+	–	–	–	–	–	
	1–100	+	–	–	–	–	–	
	1–110	+	+	+	+	–	–	
	1–120	+	+	+	+	+	+	

TABLE 3.

(Extracted from table in previous article.)

Sample	Dilution	Time of Culture Exposed to Action of Disinfectant in Minutes:						Rideal-Walker Phenol Coefficient
		2½	5	7½	10	12½	15	
Quinone	1–16,000	+	+	+	+	+	–	160 coefficient
Hydroquinone	1–200	+	+	+	–	–	–	2.5 coefficient
Phenol-quinone	1–4,000	+	+	+	+	–	–	44 coefficient

This rather heterogeneous group of substances was studied in relation to the bactericidal action of phenols colored by exposure to sunlight and the oxidation products quinol and phenol-quinol thus formed.

We were interested in the phenol coefficient four (4), for crude carbolic acid, thus confirming that obtained by the Lancet Commis-

sion and therefore admitting our results to comparison with theirs. This activity in excess of that of pure phenol is probably to be attributed, as the report of this commission pointed out, to phenoloid substances which occur in crude phenol and many coal tar disinfectants as well. The pungent odor of quinone suggested to us that of formalin which, because of very irritating effects of the latter on mucous membranes and its being a gas in watery solution, we expected to be strongly bactericidal. Our findings of its slight activity here agree with those of others. The cresols were tested because of their resemblance and relation to phenol. The coefficients 2.2 for trikresol and 2.8 for parakresol are interesting, for they disprove the statement that the mixture of the three cresols, para-, meta-, and ortho-, is more efficient than any one of them. Iodine was studied because of our interest in the recent reports about it, and because of its present wide popularity in surgical technic and surgical dressings. Its high phenol coefficient and its efficacy in killing typhoid bacilli in $2\frac{1}{2}$ minutes under the conditions of the test are further proof of its value as a practical antiseptic.

The potency of hydrocyanic acid and potassium cyanide as organic poisons and the fact that they are not oxidizing agents led us to be curious as to their capacity as disinfectants. They are seen to be less active against bacteria than is phenol.

We studied beta-naphthol in order to compare its action with that of quinone and were led to make this investigation because of the high efficiency claimed for this substance by H. C. Wood. We found that this material is not soluble in water, is soluble one part in three hundred parts of 25 per cent alcohol, forming a colorless solution, and is easily soluble in tenth normal sodium hydroxide, one part in one hundred, forming a dark yellow solution on standing. The table shows the efficiency of alcohol in strong solutions but not in weak ones and that of beta-naphthol (in alcoholic solution) in a dilution of 1–1,000, but certainly not as high as 1–2,000. The test with sodium hydroxide shows marked activity even in a dilution of 1–2,500 and also shows the interesting fact that this activity rather than being increased by a solution in it of beta-naphthol is, on the contrary, diminished. This can be attributed

to the neutralization of this substance by the weakly acid beta-naphthol, and this indicates two things: first, that the activity of the former is due to its hydroxyl group, and second, that of the latter to its acid radical. As this process of neutralization would occur in the alkaline juices of the intestine we believe that the action of this substance as an intestinal antiseptic would thereby be seriously if not entirely destroyed. Certainly with its activity cut down to that of a dilution of one to two or three hundred (see table) a sufficiently large dose could not be given to be efficient. From this consideration it would seem that beta-naphthol is certainly not an ideal substance for an intestinal antiseptic, if indeed it is at all potent in this capacity.

In light of these results, the bactericidal potency of quinone in high dilutions has suggested to us the possibility of using it as an intestinal antiseptic, because one could expect a marked action from it on the intestinal bacteria when given in the small doses in which it is necessary to administer a drug for this purpose. Similarly it would seem that it is a good agent for irrigating infected wounds, treatment of urethritis, etc. Some experiments have been begun to investigate these subjects and will be reported later. The effect of quinone on the alimentary canal of dogs will have to be thoroughly studied before one is justified in treating patients with it.

CONCLUSIONS.

1. Under the conditions of the above tests, quinone is the most efficient bactericidal substance yet reported; phenol-quinone is less active than quinone, and hydroquinone still less so.

2. Many of the commonly used disinfectants and antiseptics such as bichloride of mercury, formalin, argyrol, protargol, etc., are relatively inefficient in strengths used.

3. Iodine, alcohol in strengths above 50 per cent, silver nitrate in solution of 1–1,000 are very efficient bactericidal agents.

4. Potassium cyanide and hydrocyanic acid, although powerful organic poisons, have a weak bactericidal action.

5. The phenol coefficient of crude phenol is 4, of trikresol is 2.2, and of parakresol is 2.8, thereby disproving the statement that a

combination of all three cresols is more powerful than any one of them.

6. Beta-naphthol and sodium hydroxide neutralize one another's bactericidal action, and this would also occur in the intestine and so render the former substance useless as an intestinal antiseptic.

7. The work of recent observers and the above results indicate the advantage of getting away from the use of bichloride in surgical technic and the substitution for it of tincture iodine and 50 per cent alcohol.

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PNEUMOCOCCUS ANAPHYLAXIS AND IMMUNITY.*

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In the following pages I wish to report briefly the results of a study of anaphylaxis to pneumococcus protein and its relation to pneumococcus infection and immunity, as well as of a study of the increased reactivity of guinea-pigs to pneumococcus products and living pneumococci, as measured by the leukocytic count; and then to give a summary of a study of the effects of primary injection of certain pneumococcus extracts in NaCl solution, and a comparison of these with the symptoms produced by the injection of products obtained by the interaction of pneumococci, immune serum, and normal serum.

PNEUMOCOCCUS ANAPHYLAXIS IN SENSITIZED GUINEA-PIGS.

The view that anaphylactic intoxication in sensitized animals is due to rapid parenteral digestion of protein into toxic-cleavage products is supported strongly by recent investigations. The work of Vaughan[1] and his coworkers, of Abderhalden,[2] of Pfeiffer and Mita,[3] of Wells,[4] and others point strongly in this direction.

The protocols of the experiments on the production of active anaphylaxis are omitted, because the usual procedure, which is simple and well understood, was followed.

From a study of a long series of extracts, the following facts seem to be established: Guinea-pigs injected with dead pneumococci or pneumococcus extracts become hypersensitive to subsequent injection after a period of from eight to 12 days. It is quite immaterial whether the first injection is made subcutaneously, intravenously, intraperitoneally, or intrapleurally. The second

* Received for publication July 17, 1911.

[1] *Ztschr. f. Immunitätsf. u. exp. Therap.*, 1911, 9, p. 458.

[2] *Ztschr. f. physiol. Chemie*, 1910, 64, p. 423.

[3] *Ztschr. f. Immunitätsf. u. exp. Therap.*, 1910, 4, p. 410.

[4] *Jour. Infect. Dis.*, 1909, 6, p. 506.

dose of the clear pneumococcus extract, however, must be injected into the heart or intravenously in order to produce fatal anaphylactic shock. The symptoms produced are irritability, dyspnea, convulsions, abrupt drop in temperature and leukocytes, urination, and at times defecation. After death the lungs show the usual pallor and emphysema.

The minimum sensitizing dose of a clear pneumococcus extract in NaCl solution for guinea-pigs weighing 250 gms. is approximately 0.01 c.c. Smaller doses do not sensitize regularly. From four to seven c.c. of clear, filtered extract in salt solution, if prepared at 37° C. for 24 hours or in the ice chest for a longer period, is sufficient to cause fatal shock regularly. The injection into the left heart causes more marked symptoms than injection into the right heart, or into the jugular vein. Sensitization has been found to last as long as 410 days. The mother sensitized before or early in pregnancy transmits sensitiveness to the first litter but not to the second litter. Fresh suspensions of living pneumococci in NaCl solution do not cause fatal shock in sensitized pigs. It is necessary first to bring into solution a certain proportion of the pneumococci. The serum of sensitized guinea-pigs, when injected into a normal pig, makes the latter sensitive at the end of 12 to 24 hours (passive anaphylaxis). After severe and fatal anaphylactic shock, the serum is not toxic to normal guinea-pigs, nor does it sensitize to subsequent injection of pneumococcus extracts. Boiling pneumococci in NaCl solution and then extracting them does not interfere materially with the production of shock in sensitized pigs, nor does it destroy the sensitizing principle.

The pneumococci used in the preparation of extracts were obtained from the blood of pneumonia patients and were grown chiefly in ascites broth, broth, and on blood agar. The extracts were prepared at first by suspending approximately six to 10 billion pneumococci per c.c. of NaCl solution; later the suspensions were made so that one c.c. of the extract represented the soluble substances in approximately two to five billion cocci.

The effect of injection of pneumococcus protein brought into solution by various methods has been tested. The extracts prepared by repeatedly freezing and thawing the suspensions; pro-

longed suspension in NaCl solution in the ice chest; and those prepared at 37° C. for 24 hours or for a longer period, if non-virulent pneumococci are used, cause no symptoms on primary injection, but always in sensitized animals.

The extracts prepared from highly virulent pneumococci at 37° C. for 24 hours or longer, on the other hand, sensitize but do not always produce anaphylactic shock in sensitized pigs, and are found at times to produce symptoms in normal pigs which cannot be differentiated from typical anaphylactic shock. The extracts prepared in NaCl solution under ether at 37° C. for 48 hours and to a lesser degree those prepared over chloroform are especially apt to give primary symptoms (see Table 4). Morphologically, the pneumococci in the former methods are fairly well preserved and their affinity for basic dyes is only moderately diminished. They retain Gram's stain. In the latter, they are much disintegrated, stain faintly by basic dyes, are Gram negative, and the affinity for acid dyes is greatly increased. Extracts of pneumococci obtained from various sources, cultivated for various periods (one strain for nine years), and all grades of virulence sensitize and intoxicate with respect to each other when given in proper doses. It makes no difference whether they are cultivated in broth or on agar, whether washed or unwashed, before the extract in NaCl solution is made.

It was hoped that in the anaphylactic reaction we might find an additional test for differentiating pneumococci from *Streptococcus pyogenes*, but the experiments show that streptococcus extracts sensitize with respect to pneumococcus extracts and vice versa, while injection of streptococcus extracts rarely produce fatal shock, whether the animal is sensitized with either streptococcus or pneumococcus extracts. The symptoms are usually more marked in pigs sensitized with streptococci, and later injected with streptococcus extracts, but the difference is not great enough to be reliable. Staphylococcus extracts do not sensitize with respect to pneumococcus extracts or vice versa. Extracts of *Streptococcus mucosus* behave exactly as do those of highly virulent pneumococci.

The hypersensitive state of guinea-pigs to pneumococcus extracts disappears after intravascular injection of a large, but not

fatal, dose of pneumococcus extract, after repeated intraperitoneal or subcutaneous injections of extracts or dead pneumococci, and after recovery from a pneumococcus infection.

The question of anaphylaxis to pneumococcus extracts in relation to susceptibility and immunity to pneumococcus infection has also been studied. It has been found from numerous tests that guinea-pigs which are sensitive to pneumococcus extracts resist small injections of virulent pneumococci (from 0.01 to 0.2 c.c. of 24-hour broth culture, depending on the virulence), but are more susceptible to large injections than the normal controls. The peritoneal smears made after inoculation, and smears and plate cultures made from the blood after death bring out the interesting fact that disintegration of pneumococci (as shown by the capsular stain I have devised) in the sensitized pig is greater and the number of viable cocci in the blood after death is far less than in normal pigs. This resistance offered by the blood stream to pneumococci, from the evidence at hand, would seem to be best explained on the ground of a rapid disintegration of cocci. The promptness with which pneumococcus extracts, when autolysis is prevented, are split into toxic components in sensitized pigs, is in keeping with this idea.

Moreover, the intraperitoneal injection of a properly gauged dose of virulent pneumococci into sensitized pigs is followed in two to three hours by a sharp leukocytosis not observed in normal pigs, which succumb, while large doses produce an earlier leukopenia and death. The exudation of leukocytes into the peritoneal cavity is greater where leukocytosis is observed in the blood. The intimate relation of anaphylaxis to pneumococcus immunity is shown also by the more rapid disappearance (chiefly by phagocytosis) of non-virulent and autolyzed virulent pneumococci from the peritoneal cavity of sensitized pigs.

In other words, guinea-pigs which are hypersensitive to pneumococcus products are to a certain degree immune to pneumococcus infection, the early death of the sensitized pigs receiving the large dose being really an expression of heightened reactivity. From the study of this point, by comparing the amount of disintegration and the number of living pneumococci (as determined by the

blood-agar plate method) in the peritoneal cavity of normal and sensitized pigs, it seems certain that the pneumococci in the latter not only disintegrate more rapidly, but also multiply more rapidly, so that the early death in the sensitized animal receiving a large dose is not due wholly to the rapid destruction of the injected cocci.

In connection with active pneumococcus anaphylaxis another point of great interest should be mentioned. It has been observed many times that guinea-pigs and, to a lesser degree, rabbits, are apt to die in from eight to 12 or 14 days following the injection of relatively large doses of heated pneumococcus extracts or heat-killed pneumococci. This rarely occurs when unheated extracts in which autolysis has been carried to a more extreme point are injected, or when autolyzed pneumococci are injected. In the latter it occasionally occurs, but only when huge doses (90 billion) are introduced. It makes no difference whether the injection is made subcutaneously, intraperitoneally, or intravascularly. The time of death corresponds quite closely to the time necessary to render pigs hypersensitive. This observation may now be explained on the ground that the pneumococcus protein lodges in the tissues until antibodies are formed, and then is rapidly split into toxic products which prove fatal. The observation, which I have made many times, that animals injected with only slightly virulent living pneumococci often seem perfectly well until after seven to 10 days, and then succumb to the infection, is in keeping with this idea. Clinical observations go to show that many toxic processes of bacterial infection may be explained on the basis of anaphylaxis or allergy, as emphasized especially by von Pirquet and Schick,[1] von Pirquet,[2] and Friedberger.[3] They assume that bacteria in themselves have little or no toxic properties, and that intoxication occurs first after interaction of bacterial protein (antigen), antibody, and complement (the two latter being contained in the serum of the host) has taken place.

[1] *Wien. klin. Wchnschr.*, 1903, 16, p. 758.

[2] *Arch. of Int. Med.*, 1911, 7, p. 275.

[3] *Deut. med. Wchnschr.*, 1911, 37, p. 481.

THE INCREASED REACTIVITY OF GUINEA-PIGS AFTER INJECTIONS OF AUTOLYZED PNEUMOCOCCI.

In studying the effect of autolyzed pneumococci on intraperitoneal injection in guinea-pigs it was noted that the primary injection is not followed by leukocytosis in the peripheral circulation. A relatively slight exudation of leukocytes takes place. A second injection given from eight days to four months later is followed by a prompt intravascular leukocytosis and a more rapid exudation of leukocytes. Disappearance of pneumococci is correspondingly more rapid and takes place largely by phagocytosis. In these experiments, unless otherwise indicated, autolysis was carried out in the usual way by suspending virulent pneumococci in two changes of NaCl solution, and keeping them at 37° C. for two periods of 48 hours each. Usually chloroform was added, but pneumococci autolyzed in NaCl solution without chloroform behave similarly in this respect. It makes no difference where the first injection is made. The second injection, however, must be made intraperitoneally, intrapleurally, or into the circulation to bring out the reaction sharply. The reaction is specific.

Table 1 shows that autolyzed pneumococci cause no increase in leukocytes when injected intraperitoneally (No. 98) in normal animals. This has been found true even when the dose was made as large as 90 billion pneumococci. A second injection in from nine days to as long as four months later of the same dose is followed by a prompt leukocytosis. Clear pneumococcus extracts in this way sensitize to subsequent injections of extract as well as to autolyzed pneumococci (Nos. 127 and 44). Pneumococci autolyzed in salt solution, and then digested in pneumonic serum at 37° C. for 24 hours to four days, and then washed in salt solution, still contain the substance which sensitizes for pneumococci autolyzed in NaCl solution, but do not contain enough of the substance which produces leukocytosis when injected a second time. The clear serum, after pneumococci have been suspended in it, also contains the sensitizing principle (Nos. 92, 163, 117, and 101), but not that which causes leukocytosis. Parallel tests of the effect of normal serum show that pneumonic serum is more active, disintegration of autolyzed pneumococci being more rapid. Pneumococci auto-

TABLE 1.

Increased Reactivity of Guinea-Pigs as Manifested by Leukocytosis Following Injections of Autolyzed Pneumococci and Pneumococcus Extracts.

No. of Animal	Date and Injections	Leukocyte Count		
		Just Before	2½ Hours After	24 Hours After
93	6-30-10: 500 million pneumococci autolyzed in NaCl sol. intraperitoneally	5,200	6,000	5,800
	7-11-10: Same dose intraperitoneally	11,400	21,400	16,000
44	6-16-10: 4 c.c. ext. 143a into heart	.....	.....	.....
	6-24-10: 500 million autolyzed pneumococci intraperitoneally	9,700	17,900	13,600
119	7-18-10: 500 million autolyzed pneumococci intraperitoneally after suspension in 2 c.c. pneumonic serum at 37° C. 24 hrs. and washed in NaCl sol.	.....	.....	.....
	7-28-10: 500 million autolyzed pneumococci treated as above intraperitoneally	5,200	6,700	8,000
92	6-30-10: 500 million autolyzed pneumococci intraperitoneally	.....	.....	.....
	7-11-10: 500 million autolyzed pneumococci after treatment in 2 c.c. pneumonic serum at 37° C. 24 hrs. and washed in NaCl. sol.; intraperitoneally	10,400	8,800	13,800
163	10-8-10: 2.5 c.c. pneumonic serum after 800 million pneumococci were suspended in it for 4 days at 37° C., into heart	.....	.....	.....
	10-24-10: 500 million autolyzed pneumococci intraperitoneally	11,200	17,200	9,800
117	7-18-10: 500 million pneumococci autolyzed after suspension in pneumonic serum at 37° C. 48 hrs., washed in NaCl sol.; intraperitoneally	8,700	8,200	8,400
	7-27-10: 500 million autolyzed pneumococci intraperitoneally	7,200	18,000	9,000
155	10-3-10: 2 c.c. ext. 159 intraperitoneally	.....	.....	.....
	10-17-10: 500 million pneumococci autolyzed at 37° C. for 48 hrs. in NaCl under ether; intraperitoneally	5,400	7,200	7,600
101	7-12-10: 1 c.c. pneumonic serum (3 days before crisis) after 500 million autolyzed pneumococci suspended at 37° C. 24 hrs.; intraperitoneally	9,300	9,900	9,400
	7-23-10: 500 million autolyzed pneumococci intraperitoneally	12,000	17,400	.....
112	7-15-10: 500 million autolyzed pneumococci intraperitoneally	.....	.....	.....
	7-28-10: 500 million formalin-killed pneumococci after suspension in normal serum at 37° C. 48 hrs.; intraperitoneally	10,300	16,000	11,300
127	7-28-10: 4 c.c. ext. 144 intraperitoneally, 37° C. 24 hrs. and then on ice	.....	.....	.....
	10-24-10: Same injection intraperitoneally	10,100	20,600	15,500

TABLE 1.—*Continued.*

No. of Animal	Date and Injections	Leukocyte Count		
		Just Before	$2\frac{1}{2}$ Hours After	24 Hours After.
77......	6–16–10: 500 million autolyzed pneumococci intraperitoneally	.....	.....	.....
	6–24–10: 500 million autolyzed pneumococci intraperitoneally	14,800	22,000	20,900
	7–28–10: 4 c.c. ext. 144 into left heart*	12,800	4,300	13,200
	8–18–10: 500 million autolyzed pneumococci intraperitoneally	9,400	8,200	8,900

* Marked symptoms of anaphylaxis.

lyzed in NaCl solution under ether 48 hours lose much of the part which causes leukocytosis (No. 155), the cocci here, like those after treatment in serum, being more disintegrated. Heat and formalin (2 per cent in NaCl solution) killed pneumococci do not produce leukocytosis either on first or second injection, while partially serum-digested, formalinized, and heat-killed pneumococci produce leukocytosis in sensitized pigs. The fact that well preserved dead pneumococci do not produce leukocytosis is probably not due to their relative insolubility or greater resistance to phagocytosis, but rather to a substance which they contain which depresses the leukocytic mechanism.

During the disintegration of pneumococci, whether by the action of their own proteolytic ferment in salt solution or by the digestive action of serum, there goes into solution early a toxic substance (pneumococcus-anaphylatoxin) which lowers the temperature and opsonic index, and when injected in proper dosage depresses the leukocytic mechanism. There remains in the partially digested cocci a substance which produces a sharp leukocytosis when injected into sensitive pigs, and an abrupt rise in opsonin in man. Finally, on further digestion, both these substances are destroyed. It would seem that the leukopenia in overwhelming pneumococcus infection, the hyper-leukocytosis in severe but more favorable infections, and the slight leukocytosis in the mild cases may be explained on the basis of these results.

The experiments on guinea-pigs (No. 77) show the usual leukocytosis on second injection of autolyzed pneumococci; a leukopenia and severe anaphylactic shock on intravascular injection of extract

(prepared so that little digestion takes place); and an absence of leukocytosis on injection of autolyzed pneumococci following this reaction. In other words, the reactivity of guinea-pigs to pneumococcus extracts and autolyzed pneumococci as measured by leukocytosis runs a course quite similar to that of sensitized pigs as measured by the symptoms and temperature. Moreover, the results obtained by this method support the idea that anaphylactic symptoms in sensitized animals are due to a parenteral digestion of protein, because autolyzed pneumococci are digested and destroyed more rapidly in sensitized pigs when leukocytosis is produced. This fact is shown strikingly in No. 77, the pneumococci disappearing most rapidly after the second injection which was followed by an increase of leukocytes.

ANAPHYLACTIC INTOXICATION OF NORMAL GUINEA-PIGS WITH PRODUCTS OBTAINED BY THE INTERACTION OF PNEUMOCOCCI, IMMUNE SERUM, AND NORMAL SERUM.

Friedberger[1] and Friedeman[2] were the first to obtain a highly toxic substance capable of causing the symptoms of anaphylaxis by the interaction of albumen or bacteria (antigen), immune serum (amboceptor), and normal serum (complement). The former concludes that the symptoms in infectious diseases are due to "anaphylatoxin," a split product of the various bacterial proteins. The existence of a separate poison for various bacteria, he says, is not proven nor necessary to explain the facts, the difference in the character of the infection by the different species being due, not to differences in bacterial proteins, but to different modes of attack on part of the host.

Neufeld and Dold[3] produced primary anaphylaxis in guinea-pigs by treating pneumococci, typhoid bacilli, or cholera vibrio with the corresponding immune serum, and later with complement. They believe that bacteriolysis prevents the formation of anaphylatoxic substance, because pneumococci are well preserved when the mixture produces fatal intoxication, and because they were able to

[1] *Deut. med. Wchnschr.*, 1911, 37, p. 481.

[2] *Ztschr. f. Immunitätsf. u. exper. Therap.*, 1909, 2, p. 591.

[3] *Berl. klin. Wchnschr.*, 1911, 48, p. 55.

get intoxication with cholera vibrio only when bacteriolysis was prevented by placing the mixture on ice. They are not certain whether the bacteria furnish the mother substance, but conclude that the three components, antigen, amboceptor, and complement, are essential to produce primary anaphylaxis.

More recently Wasserman and Keysser[1] conclude that anaphylatoxin is not necessarily a split product of the bacterial antigen because barium sulphate, kaolin, and other inert substances act as well as bacteria with amboceptor and complement. They believe that the amboceptor furnishes the mother substance of the anaphylatoxin, and name the poisonous substance "toxopeptid," assuming that it is an early product of the ferment action of complement on amboceptor and not on antigen. They conclude that the bacterial antigen or barium sulphate alters the surface tension and adsorption properties, so as to favor the digestion of amboceptor by complement. Friedberger[2] considers, however, that the horse serum used by them as amboceptor acted in reality as antigen, the guinea-pig serum containing both complement and amboceptor. They showed that their mixtures are harmless when first made, very toxic at a certain period, and again harmless on longer treatment at 37° C.

As shown by Table 2, symptoms of anaphylactic intoxication may be obtained in normal guinea-pigs with pneumococci treated in various ways:

1. By the action of pneumococci of immune pneumococcus serum, immune streptococcus serum, humanized rabbit serum, heated normal goat serum followed with fresh normal guinea-pig serum (Table 2, experiments 1–12).

2. By treating pneumococci in ether or soap for a short time, or in NaCl solution at 37° C. for a longer period, instead of immune serum, and then suspending them in guinea-pig complement (Table 2, experiments 18, 21, 25). Ether-treated and soaped pneumococci also yield the substance if treated in immune pneumococcus serum and then in complement (Table 2, experiments 17 and 20).

[1] *Folia Serologica*, 1911, 7, p. 243.

[2] *Ztschr. f. Immunitätsf. u. exp. Therap.*, 1911, 9, p. 234.

TABLE 2.

ANAPHYLACTIC INTOXICATION OF NORMAL GUINEA-PIGS PRODUCED BY PNEUMOCOCCI UNDER VARIOUS CONDITIONS.

Experiment	Pneumococci	Serum Treatment of Pneumococci		Symptoms	Appearance of Pneumococci at Time of Injection
		Immune Sera	Complement		
1	Virulent pneumococci from 30 c.c. broth culture	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Death in 3 min.	Mostly Gram positive
2	Non-virulent pneumococci from 30 c.c. broth culture	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Death in 4 min.	Mostly Gram positive
3	Autolyzed pneumococci	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	No symptoms	Dissolved
4	Boiled virulent pneumococci from 30 c.c. broth	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Moderate symptoms	Largely Gram positive but a fair number were negative
5	Boiled virulent pneumococci from 30 c.c. broth	1 c.c. pneumococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 72 hrs.	Marked symptoms	Largely Gram positive but a fair number were negative
6	Virulent pneumococci from 30 c.c. broth culture	1 c.c. pneumococcus immune serum at 37° C. 1 hr.	4 c.c. in ice chest 48 hrs.	Death in 2 min.	Largely Gram positive but a fair number were negative
7	Virulent pneumococci from 30 c.c. broth culture	1 c.c. streptococcus immune serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Death in 2 min.	Largely Gram positive but a fair number were negative
8	Virulent pneumococci from 30 c.c. broth culture	1 c.c. humanized rabbit serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Death in 4 min.	Largely Gram positive but a fair number were negative
9	Virulent pneumococci from 30 c.c. broth culture	1 c.c. fresh normal rabbit serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	No symptoms. No drop in temperature	Largely Gram positive but a fair number were negative
10	Virulent pneumococci from 30 c.c. broth culture	1 c.c. pneumonic serum at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Marked symptoms but recovered	Largely Gram positive but a fair number were negative

11	Virulent pneumococci from 30 c.c. broth culture	3 c.c. normal guinea-pig serum	4 c.c. in ice chest 48 hrs.	No symptoms	Largely Gram positive but a fair number were negative
12	Virulent pneumococci from 30 c.c. broth culture	1 c.c. normal goat serum free from complement at 37° C. ½ hr.	4 c.c. in ice chest 48 hrs.	Marked symptoms but recovered	Largely Gram positive but a fair number were negative
13	Virulent pneumococci from 30 c.c. broth culture	....	4 c.c. NaCl sol. in ice chest 48 hrs.	No symptoms	Largely Gram positive but a fair number were negative
14	....	....	4 c.c. normal guinea-pig serum in ice chest 48 hrs.	No symptoms	....
15	....	....	4 c.c. normal guinea-pig serum 37° C. 24 hrs., then in ice chest 24 hrs.	Severe symptoms. Drop of 5° F.	....
16	....	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs. and then at 37° C. 18 hrs.	Death in 4 min. from typical symptoms. Lungs emphysematous	....
17	Virulent pneumococci from 30 c.c. broth treated with ether for ½ hr.	1 c.c. pneumococcus immune serum for ½ hr. at 37° C.	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	Death in 2 min. from typical symptoms. Lungs emphysematous	Mostly Gram negative
18	Virulent pneumococci from 30 c.c. broth treated with ether for 40 min. at 37° C.	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	Death in 2 min. from typical symptoms. Lungs emphysematous	Mostly Gram negative
19	Virulent pneumococci from 30 c.c. broth	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	No symptoms	Mostly Gram positive
20	Virulent pneumococci from 30 c.c. broth treated in 1–2,500 sod.-oleate at room temp. 1 hr.	1 c.c. pneumococcus immune serum in ice chest 24 hrs.	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	Death in 3 min. Typical symptoms. Lungs emphysematous	Mostly Gram negative
21	Virulent pneumococci from 30 c.c. broth treated in 1–2,500 sod.-oleate room temp. for 1 hr.	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	Severe symptoms. Nearly dead in 10 min. Drop of 2.5° F.	Mostly Gram negative
22	Virulent pneumococci from 30 c.c. broth treated with 1 c.c. human bile at 37° C. for ½ hr.	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	No symptoms	All Gram negative and practically all dissolved

TABLE 2—*Continued.*

Experiments	Pneumococci	Serum Treatment of Pneumococci		Symptoms	Appearance of Pneumococci at Time of Injection
		Immune Sera	Complement		
23	Virulent pneumococci from 30 c.c. broth treated in 1–1000 sod.-oleate room temp. for $1\frac{1}{2}$ hrs.	....	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	No symptoms	All Gram positive and practically all dissolved
24	Virulent pneumococci from 30 c.c. broth treated in 25 per cent phenol at 37° C. 1 hr.	1 c.c. pneumococcus immune serum in ice chest 20 hrs.	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	Marked symptoms. Drop of 3.7° C. in 10 min.	All Gram positive and practically all dissolved
25	Virulent pneumococci from 30 c.c. broth treated in 1 c.c. NaCl at 37° C. 24 hrs.	....	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	Death in 4 min. Typical symptoms. Lungs emphysematous	Mostly Gram negative but disintegration not marked
26	3 c.c. ext. in NaCl from pneumococci in 30 c.c. broth at 37° C. 48 hrs.	....	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	Death in 2 min. Typical symptoms. Lungs emphysematous	....
27	3 c.c. NaCl	....	4 c.c. normal guinea-pig complement in ice chest 24 hrs.	No symptoms	....
28	Virulent pneumococci from 30 c.c. broth	1 c.c. pneumococcus immune serum 37° C. for $\frac{1}{2}$ hr.	4 c.c. normal guinea-pig complement in ice chest 48 hrs.	Death in 2 min.	Mostly Gram negative
29	Virulent pneumococci from 30 c.c. broth	1 c.c. pneumococcus immune serum 37° C. for $\frac{1}{2}$ hr.	4 c.c. normal guinea-pig complement heated 58° C. for $\frac{1}{2}$ hr.	Death in $3\frac{1}{2}$ min.	Mostly Gram negative
30	Virulent pneumococci from 30 c.c. broth	1 c.c. pneumococci immune serum 37° C. for $\frac{1}{2}$ hr.	4 c.c. normal guinea-pig complement heated 58° C. for $1\frac{1}{2}$ hrs.	Severe symptoms but recovered	Mostly Gram negative

NOTE.—The pneumococci were treated in immune serum as indicated, the latter removed and the treated cocci suspended in normal guinea-pig serum (15° C.) for the time given. Control tests showed that it makes no difference whether the pneumococci are removed before injection or not. Injections of guinea-pig complement alone after being kept in the ice chest never produced symptoms in my hands. The Gram stain is a good index of the degree of disintegration at the time of injection. All injections in Table 2 were made into the jugular vein.

3. By suspending pneumococcus extracts (in which autolysis has not been carried far enough to produce intoxication) in guinea-pig complement at 15° C. 24 hours (Table 2, experiment 26).

Identical symptoms may be obtained with normal guinea-pig serum alone if kept at 37° C. for the proper length of time (Table 2, experiments 15 and 16).

The intoxicating substance obtained by the interaction of pneumococci, immune serum, and complement is thermolabile (Table 2, experiments 28, 29, 30).

Pneumococci from which the substance has been extracted in salt solution, those dissolved in bile and by strong solutions of soap (sodium-oleate 1–1,000) no longer yield any symptoms when treated in immune serum and complement or in the latter alone (Table 2, experiments 3, 22, 23).

Boiled virulent pneumococci, non-virulent pneumococci, phenol (0.25 per cent), and chloroform-treated virulent cocci give the substance with more difficulty in immune serum and complement (Table 2, experiments 2, 4, 5, 24). In other words, pneumococci, which we may assume contain an autolytic ferment, produce the anaphylatoxic substance with greatest ease in the mixture with serum. Morphological and tinctorial changes in the pneumococci indicate that the cocci themselves contribute a part at least of this substance, the contrary view of Wasserman and Keysser notwithstanding. In the salt solution extract (see below) they of course are the only source of the poisonous substance.

That the substance in various serum mixtures, which produces anaphylactic intoxication in normal pigs, is an early split product of protein is indicated in Table 3. The proteolytic enzymes in normal untreated serum undoubtedly play a rôle in producing the anaphylatoxin. But the proteolytic enzyme contained in virulent pneumococci also plays a rôle, since, when the latter are added, digestion is carried to the proper point at a lower temperature and (as shown in other experiments) in four hours at 37° C. instead of in 24 hours as required for the serum alone.

The results of a study of the opsonic index to pneumococci and streptococci, just before and shortly after injection of the various mixtures containing pneumococci, show that 10 minutes after death

there is no measurable drop in the index in the animals which die in two to four minutes. The serum of those which have severe symptoms but recover show a marked drop in about 15 minutes after the height of the shock, while those in which no symptoms occur show no change in the index. The loss of opsonin is specific for pneumococci. This is of interest especially in connection with the fact that the protein of pneumococci and streptococci are so nearly alike that both sensitize pigs for the other.

TABLE 3.

Comparison of the Effect on Normal Guinea-Pigs and the Amount of Proteolysis in Various Serum Mixtures.

Experiment	Material Injected (4 c.c. in Each Experiment Intravenously)	Formalin Titration	Symptoms in Normal Guinea-Pigs
1.......	Normal guinea-pig serum	0.4 2.2	No symptoms
2.......	Normal guinea-pig serum at 37° C. for 24 hrs.	0.45 2.6	Death from typical symptoms in 3 min.
3.......	Normal guinea-pig serum heated 60° C. for 1 hr.	0.4 2.0	No symptoms
4.......	Normal guinea-pig serum 15° C. 48 hrs.	0.5 2.3	No symptoms
5.......	Normal guinea-pig serum at 15° C. 48 hrs., after heating 60° C. 1 hr.	0.2 2.2	No symptoms
6.......	Normal guinea-pig serum and pneumococci from 30 c.c. broth after treatment in immune serum for ½ hr. at 37° C. at once	0.3 2.2	No symptoms
7.......	Normal guinea-pig serum and pneumococci from 30 c.c. broth after treatment with immune serum for ½ hr. at 37° C., 15°, 48 hrs.	0.6 2.4	Death in 3 min.
8.......	Normal guinea-pig serum heated and pneumococci from 30 c.c. broth after treatment in immune serum for ½ hr. at 37° C. at once	0.5 2.4	No definite symptoms but very ill
9.......	Normal guinea-pig serum unheated and pneumococci from 30 c.c. broth treated in ether 37° C. 40 min.	0.6 2.5	Death in 3 min.

The drop in the opsonic index is roughly proportionate to the severity of the symptoms if death is not immediately fatal. It makes no difference whether they are produced in sensitized pigs by the injection of pneumococcus extracts in which autolysis has been largely prevented; in normal pigs with extracts of pneumococci in which autolysis has gone to the proper point; or by the injection of the various serum mixtures, serum broth alone, and serum-broth cultures. In one series of seven experiments the

content in hemolytic complement just before and after the injections was tested and a loss was noted in all, even when no symptoms of anaphylaxis occurred. This, however, is greatest when the loss in specific opsonin is most marked.

INTOXICATION WITH EXTRACTS OF PNEUMOCOCCI IN SALT SOLUTION.

As shown in Table 4, symptoms and postmortem findings following the injection of extracts of pneumococci prepared under ether in NaCl solution at 37° C. for the proper length of time, which varies with different strains, are identical with those observed in sensitized pigs following the injection of pneumococcus extracts in which autolysis has been largely prevented. Extracts of virulent pneumococci prepared in the cold, at 37° C. for 24 hours, of boiled virulent pneumococci, and of non-virulent pneumococci at 37° C., both with and without ether or chloroform, do not produce definite symptoms resembling anaphylactic shock in normal pigs. That the toxic substance is an early split product of the pneumococcus protein seems certain because trypsin destroys it (Table 4, Nos. 341 and 342). The formalin titration method[1] of Henriques and Sörensen[2] shows only a slight splitting when symptoms were produced and a greater splitting when trypsin was added and no symptoms were produced. The early toxic effects disappear (Table 4, Nos. 349, 355, 359) on longer residence of the pneumococci at 37° C. in NaCl solution under ether, and finally, no symptoms can be obtained with extracts of non-virulent pneumococci (no autolytic ferment) nor of boiled virulent pneumococci, when the proteolytic enzyme is destroyed.

The amount of protein in the NaCl extracts as compared with serum extracts is exceedingly small—so small that only slight differences are obtained when titrated for proteolysis by the formalin

[1] (1) To 10 c.c. of the salt-solution extract or serum mixtures are added 25 c.c. of distilled water and 10 drops of phenolphthalein. This is brought to a permanent pink blush with N/10 KOH. (2) To 5 c.c. of formalin are added 10 c.c. water and the same indicator. This is also brought to a permanent pink. Add 1 to 2 and titrate to a deep red. The first figures given in the tables represent in cubic centimeters the amount of N/10 KOH required to neutralize 10 c.c. extract; the second, the amount required to bring to a deep red after formalin was added.

I am aware of the fact that in the presence of carbonates and phosphates such as occur in serum and broth, the end point of this reaction is somewhat indefinite, but since comparative results only are used, this objection, which might be raised, does not hold. Moreover, precipitating out the carbonates and phosphates gives similar results.

[2] *Ztschr. f. physiol. Chemie*, 1909, 63, p. 27.

TABLE 4.

Production of "Anaphylactic Shock" in Normal Guinea-Pigs with Pneumococcus Extracts in NaCl Solution.

No. of Animal	Size of Injection	No. and Preparation of Extract. Place of Injection	Result
341	4 c.c.	Ext. 267*b* prepared in NaCl with chloroform at 37° C. for 24 hrs. Into left heart	Typical symptoms. Death in 10 min. Drop of 3° F. in 10 min. Lungs emphysematous
342	4 c.c.	Ext. 267*a* prepared in NaCl with chloroform and 1–1,000 trypsin. Into left heart. At 37° C. for 24 hrs.	No symptoms
472	7 c.c.	Ext. of virulent pneumococcus 587 in NaCl under ether at 37° C. 48 hrs. Into jugular vein	Typical spasms and dyspnea. Death in 5 min. Drop of 5° F. Lungs emphysematous
472*b*	7 c.c.	Ext. of pneumococcus 575 in NaCl under ether at 37° C. 48 hrs. Into jugular vein	Typical symptoms. Death in 5 min. Drop of 5° F. Lungs emphysematous
479	7 c.c.	Ext. of virulent pneumococcus 587 in NaCl under ether at 37° C. 48 hrs. and heated to 60° C. for ½ hr. Into jugular vein	Some dyspnea, no spasms. Drop of 1° F. in ½ hr. Recovered
475	7 c.c.	*Ibid.*, but after boiling for 10 min. Into jugular vein	No symptoms, drop of 0.5° F. in ½ hr. Remained well
495	7 c.c.	NaCl and ether. Ether removed by passing stream of air through fluid. Into jugular vein	No symptoms. Drop of 0.8° F. in 1 hr.
161	3.5 c.c.	Ext. prepared from virulent pneumococcus in NaCl under ether at 37° C. for 4 days. Into left heart	Profound prostration. Dyspnea 30 sec. after injection, lasting 30 min. Severe chills after 1 hr. Died during night. Autopsy showed ulcer of stomach. Cultures sterile from heart's blood
148	3.5 c.c.	Ext. 162 prepared in NaCl under ether at 37° C. for 4 days. Into left heart	Marked symptoms at once. Died in 11 min. Lungs emphysematous
150	3.5 c.c.	Ext. 164 prepared in NaCl under ether at 37° C. for 48 hrs. Into left heart	Marked symptoms in 4 min. dyspnea, convulsive twitchings, death in 15 min. Moderate pulmonary emphysema
149	3.5 c.c.	Ext. 162 prepared in NaCl under ether at 37° C. for 4 days. Into right ventricle	No definite symptoms, but death during night. Autopsy negative
226	3.5 c.c.	Ext. of virulent pneumococci in NaCl under ether at 37° C. 48 hrs. Into left heart	Severe symptoms at once. Prostration, dyspnea. Died during night. Drop of 3° F. in 1 hr.
222	5 c.c.	Ext. 189*c* prepared in NaCl under ether at 37° C. 48 hrs. Into left heart	Severe symptoms. Labored respiration, spasms, great irritability, but recovered. Temp. dropped 3° F. in 1 hr.
185	6 c.c.	Ext. 171*b* prepared in NaCl under ether at 37° C. for 3 days. Into left heart	Moderately severe symptoms. Leukocytes before injection, 7,200. Leukocytes 1 hr. after injection, 4800

TABLE 4.—*Continued.*

No. of Animal	Size of Injection	No. and Preparation of Extract. Place of Injection	Result
349	4 c.c.	Ext. 269 prepared in NaCl under ether at 37° C. for 24 hrs. Into left heart	Severe symptoms. Drop of 2° F.
355	4 c.c.	Ext. 269 37° C. 48 hrs. Into left heart	No symptoms. Slight rise in temperature
359	4 c.c.	Ext. 269 37° C. 96 hrs. Into left heart	No symptoms of anaphylactic shock. Rise of 2° C. in 1 hr.

NOTE.—Precipitation methods and the formalin-titration method prove that proteolysis was fairly complete where trypsin was added and slight where chloroform only was added (see 341).

method. The extracts which produce primary symptoms show slight splitting; extracts prepared on ice which give no symptoms show no splitting; while those which do not produce symptoms after long residence at 37° C. show the most. The differences, however, are near the border line of experimental error.

The differences in effect on the temperature in normal pigs of extract prepared at 37° C. for varying periods is also shown in Table 4 (Nos. 349, 355, 359); early, when the toxicity is greatest, a drop in temperature occurs, while later the same quantity of extract produces a rise. The toxic substance here, as in serum mixtures, is destroyed largely on heating to 60° C. for $\frac{1}{2}$ hour, and wholly on boiling for 10 minutes (No. 475). The observation that the toxic substance is so easily destroyed by heat is in harmony with the fact that in order to obtain typical symptoms with pneumococcus extracts it is necessary to interrupt autolysis at a certain time.

The extracts of virulent pneumococci which produce symptoms and those which do not because of too long exposure to 37° C., do not produce anaphylaxis in sensitized pigs. In the case of the active extracts this might be explained, with Friedberger and others, on the ground that the proteolytic enzyme contained in the sensitized pig rapidly splits the poison into non-toxic material. In view of the fact that sensitized pigs are relatively immune to small doses of virulent pneumococci, it might be also that the serum of the sensitized pig contains a substance that neutralizes the pneumococcus poison.

Since pneumococcus extracts prepared under ether give anaphylactic symptoms in normal guinea-pigs most regularly, it was necessary to determine whether or not this, in part, is due to the chemical action of the ether on the pneumococci or whether ether only favors the action of the autolytic ferment. Accordingly, experiments were made with two strains of virulent pneumococci. In each case a large quantity of cocci was suspended in NaCl solution thoroughly mixed and divided into two equal parts. Parts *A* were boiled for 10 minutes, then ether added; to parts *B*, ether was added without boiling; all were now placed at 37° C. for 48 hours. At the end of this time the pneumococci were centrifuged, the ether removed, and the toxicity and other properties of the supernatant fluid tested by intravenous injections. The portions boiled before adding ether produced no symptoms, the portions not boiled caused typical symptoms of anaphylactic shock. Formalin titration of the former showed it to be unchanged, while the latter require 0.3 c.c. N/10 KOH to produce pink to phenolphthalein per 10 c.c. of extract after formalin is added.

The amount of coagulable material in my salt solution pneumococcus extracts is so small as compared with the amount of peptone necessary to produce fatal intoxication that its action cannot be due to peptone alone because Kraus and Biedl[1] found that 3 to 4 c.c. of a 10 per cent solution of Witte peptone were necessary to cause fatal intoxication in guinea-pigs weighing 250–300 gms. This is in accord with the work of Spiro and Pick[2] and Papielski,[3] who find that the intoxication following the injection of peptone is not due to peptone, but to other substances present. When viewed from the amount of coagulable substance, phosphotungstic acid producing only a slight turbidity, the material obtained from virulent pneumococci is exceedingly toxic. The results of the formalin titration indicate that this toxic substance is an earlier split product of pneumococci than the peptone stage, and that when this is reached the toxicity disappears.

In order to study more accurately the degree of proteolysis as

[1] *Wien. klin. Wchnschr.*, 1909, 22, p. 363.

[2] *Ztschr. f. physiol. Chemie*, 1900, 31, p. 235.

[3] *Pflüger's Arch.*, 1909, 126, p. 483.

determined by the formalin-titration method, in relation to the symptoms produced in normal pigs, further experiments (Table 5) were made with serum and serum-broth cultures. Here protein splitting is more marked because of a larger amount present than the extract in salt solution. The ability of the serum-broth mixtures

TABLE 5.

EFFECT OF SERUM BROTH AND BROTH ON GUINEA-PIGS IN RELATION TO INTOXICATION PRODUCED BY PNEUMOCOCCI.

Experiment	Normal Guinea-Pig Serum One Part, Meat Dextrose 2 per cent, Broth Nine Parts	Formalin Titration	Symptoms in Normal Guinea-Pigs. 4.5 c.c. Intravenously
1......	Serum broth. Im.	0.8 3.4	No symptoms
2......	Serum broth at 37° C. 24 hrs.	1.0 4.6	No symptoms
3......	Serum broth and pneumococci from 30 c.c. broth culture at 37° C. for 4 hrs.	2.9 5.0	No symptoms
4......	Serum broth and pneumococci from 30 c.c. broth culture at 37° C. for 24 hrs.	2.6 5.8	Death in 4 min.
5......	Serum broth and pneumococci from 30 c.c. broth culture at 37° C. for 48 hrs.	3.0 5.8	Very severe symptoms
6......	Serum broth and pneumococci from 30 c.c. broth culture at 15° C. for 48 hrs.	3.0 5.4	Moderate symptoms
7......	Serum broth and pneumococci from 30 c.c. broth culture at once	1.5 4.4	No symptoms
8......	Serum broth at 37° C. 48 hrs.	1.1 4.4	Slight symptoms
9......	Serum broth at 37° C. 96 hrs.	1.6 6.2	Profound symptoms, nearly died
10......	Meat dextrose 2 per cent broth at 37° C. for 96 hrs.	0.8 2.45	No symptoms
11......	Meat dextrose 2 per cent broth after highly virulent pneumoccci are grown in it for 96 hrs.	3.2 5.2	Moderately severe symptoms

to produce intoxication with symptoms like those of anaphylaxis bears a close relation to the degree of proteolysis. It makes no difference, however, whether the splitting is brought about by the complement in the serum broth alone (Table 5, experiments 1, 2, 8, and 9), by longer residence at 37° C., or earlier by complement, aided by the proteolytic enzyme contained in the virulent pneumococci (experiments 3, 4, 5, and 6), or finally by the latter alone on longer exposure to 37° C. in broth alone (experiments 10 and 11).

SUMMARY.

Anaphylaxis to pneumococcus protein does not differ materially from that to other proteins.

When pneumococcus extracts (antigen), in which autolysis has not gone too far, are injected into sensitized animals, there occurs a rapid splitting into a toxic substance.

In the sensitized guinea-pig, autolyzed and heat-killed cocci as well as living non-virulent and virulent pneumococci are dissolved more rapidly than in the normal animal. The relatively slight yet definite immunity of sensitized guinea-pigs to virulent pneumococci is probably largely due to this cause.

It is possible to obtain from virulent pneumococci by autolysis in NaCl solution a substance which produces symptoms, fatal and otherwise, wholly like those of anaphylaxis in normal guinea-pigs, just as from mixtures of pneumococci and serum. This substance is probably an early split product of pneumococcus protein and in its action is identical with that obtained with the mixture of pneumococci and serum.

Autolysis of normal serum, digestion in serum-broth mixtures, and digestion of pneumococcus protein in serum-broth and broth cultures yield a substance or substances with the same effect. In all these cases, it seems to concern a proteolytic action. When this is comparatively slow a higher temperature for a longer time yields the same result as the more active process at a lower temperature.

Pneumococcus anaphylatoxin depresses the leukocytic mechanism. Autolyzed pneumococci, from which this substance has been removed, on the contrary, have the power of stimulating the leukocytic mechanism in sensitized guinea-pigs. Further digestion in serum or in salt solution under ether destroys this property also. This affords the best explanation we have (1) of the development of leukopenia in overwhelming experimental pneumococcus infections, and in lobar pneumonia in man, when digestion of pneumococci, we may assume, is imperfect and there is liberated a large amount of anaphylatoxin; (2) of the high leukocytosis in severe but more favorable infections where digestion is carried rapidly to a farther point, and there is liberated a relatively large amount of

the latter substance which stimulates the leukocytic mechanism and (3) of the slight leukocytosis in the mild infections where digestion of pneumococci is still more rapid, and where both anaphylatoxin and the leukocytogenic substance are rapidly digested into relatively harmless products.

The action of pneumococcus anaphylatoxin is identical with other protein anaphylatoxins, but when considered from the standpoint of the amount of precipitable substance contained in pneumococcus extracts, pneumococci yield a very much larger proportion of this cleavage product than does serum or egg white during digestion with serum.

The fact that virulent pneumococci have within themselves a proteolytic enzyme which splits their protein into a highly toxic substance is strong indication that certain strains of pneumococci may cause infection forthwith without first rendering the host allergic. This is quite in keeping with the fact that in pneumococcus infections an incubation period is not an invariable rule. On the other hand, in certain instances, a previous sensitization before severe symptoms set in probably occurs. This might well be the case in lobar pneumonia when the chill occurs a week or 10 days after the patient contracted a severe cold or bronchitis. The distribution by lobes in typical cases may be related to the bronchial spasm which this toxic substance produces. The early dyspnea and increased respiration before consolidation is demonstrable is in keeping with this idea.

The fact that the allergic state or hypersensitiveness in guinea-pigs to pneumococcus extracts disappears after repeated inoculations of dead pneumococci or pneumococcus extracts with severe anaphylactic shock, and after recovery from experimental pneumococcus infections, suggests the possibility that the crisis in lobar pneumonia, for example, is a reaction against anaphylatoxin either in the nature of the development of an anti-anaphylatoxin, or what seems more likely, by the development of enzymes, to a point where rapid splitting of anaphylatoxin into non-toxic products occurs.

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FURTHER OBSERVATIONS ON THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF LUES IN THE MILITARY SERVICE: AN ANALYSIS OF 3,950 TESTS.*†

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In the *Journal of Experimental Medicine* (1910, 12, p. 726), I published the results obtained from the use of the Wassermann complement fixation test in the diagnosis of lues in the army, giving the data of the cases examined to September 10, 1910, the method having been adopted in this laboratory November 1, 1909. In this paper I desire to give the results we have obtained with this test from November 1, 1909, to June 30, 1911, believing that the large number of cases discussed proves conclusively the great value of the test in the diagnosis of lues both in the military service and civil life.

Technic.—The technic employed for the complement fixation test in this laboratory is practically that recommended by Noguchi, the only departure from his technic being the use of an alcoholic extract of fetal luetic liver as antigen, and the use of inactivated

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sera. It is impossible for us to use active serum as we make tests for army posts situated at some distance from the laboratory, in consequence of which most of the serum is from two to three or four days old before it can be tested. For this reason we have preferred to use an alcoholic extract of luetic liver as antigen instead of the acetone-insoluble fraction recommended by Noguchi, and in order to avoid the thermolabile anticomplementary bodies and the non-specific reactions sometimes observed when an alcoholic extract is used as antigen, the sera are always inactivated just before making the test. I have compared this method in a large number of cases with the acetone-insoluble antigen of Noguchi, using inactivated sera, and have found that the results are identical except in cases giving a weak plus-minus reaction, when the acetone-insoluble fraction gave a slightly larger percentage of positive results. However, as such reactions are of little value in diagnosis we have seen no reason to abandon the use of the alcoholic extract when inactivated serum is used. If it were possible for us to use active serum, the acetone-insoluble fraction would give slightly better results than the alcoholic extracts, as has been shown by numerous investigators.

Before adopting the complement fixation test as a method of diagnosis of lues in the army, careful experiments were conducted in regard to the keeping qualities of human sera, as some authorities had reported that certain specimens of normal serum, if kept for several days, would give a positive reaction.

In all, 50 normal sera were tested, the blood being collected in Wright tubes and the serum allowed to separate, after which it was pipetted into suitable glass containers and sealed. Enough of these tubes were prepared to enable us to make a complement fixation test upon each serum at intervals of one week, for a month. Of the 50 sera examined, all gave a negative reaction at the time of collection and 43 were still negative at the end of a month, while seven had developed anticomplementary bodies which produced complete inhibition of hemolysis in both antigen and control tubes. As an alcoholic extract was used as antigen the sera were always inactivated before the test was made, but in two sera tested before inactivation, both being about two days old, a positive reaction was

obtained. Both of these sera gave a negative reaction after inactivation, and neither was contaminated by bacteria. This observation confirms that of other investigators who have found that non-specific reactions may occur in testing active sera when an alcoholic extract is used as antigen.

The experiments have convinced us that as long as normal sera remain free from bacterial contamination, and are inactivated before use, they do not give a positive reaction, even though kept at room temperature for a month. However, in some of the sera, both thermolabile and thermostabile anticomplementary bodies developed which caused inhibition of hemolysis in both antigen and control tubes. The experiments proved that a positive reaction cannot occur in normal sera, if proper precautions are taken, for a period of time far in excess of any delay that may occur before the specimens reach the laboratory.

As stated, the antigen employed has been an extract of luetic liver in absolute alcohol. The crude extract, prepared according to Noguchi, is diluted just before use with nine parts of normal salt solution, and of this 0.1 c.c. is used for each tube, provided this dose is found sufficient after titration of the diluted antigen. I have used a large number of antigens prepared from normal tissues but have not found any as satisfactory as those prepared from fetal luetic liver. It should be remembered that in using an alcoholic extract as antigen the serum to be tested *must* be inactivated.

The blood suspension used is a one per cent suspension of human red blood corpuscles prepared as recommended by Noguchi.

The amboceptor is prepared by immunizing rabbits to human red blood corpuscles in the manner recommended by Noguchi. When the serum has been found by titration to be of sufficient strength the animals are bled and filter paper is impregnated with their serum. Thus preserved the serum keeps very much longer than when it is used in the fluid condition, and I have found that amboceptor paper loses but little strength even for periods as long as six months. The paper should be titrated at least every two weeks in order to be sure of its exact strength.

The complement used has been guinea-pig serum not over 24 hours old. Serum kept from 12 to 20 hours is stronger in comple-

ment than fresh serum or serum over 20 hours old. I desire to emphasize strongly the necessity of titrating the complement before using it, for guinea-pig serum varies considerably in complementary qualities. It is also necessary to use the serum of at least two pigs, as occasionally the serum of one pig is so weak in complement that it cannot be used for the test.

The method of making the test will not be discussed as it is fully described in the work of Noguchi upon the serum diagnosis of syphilis.

General results.—The tests made in this laboratory may be divided into the following three classes: *Group 1:* Cases in which the clinical diagnosis was lues or suspected lues, or in which there was a history of previous infection (this includes latent cases). *Group 2:* Cases in which clinical symptoms or diagnosis were of some other disease. *Group 3:* Normal individuals.

The total number of individuals examined to June 30, 1911, has been 2,435, while 1,515 repetitions of the test have been made as a guide to treatment, making a total of 3,950 tests. In addition to these, several hundred tests have been made in research work upon the subject of complement fixation. It is believed that the value and accuracy of the technic employed is amply demonstrated by the results obtained in this large series of cases, especially as these results were controlled by repetitions of the test in over 500 patients who were placed upon treatment after the primary test.

Of the 2,435 individuals tested, 1,322 gave a positive reaction, and 1,113 a negative reaction. In the following pages the conventional signs ++, +, and +− are used to indicate varying degrees of inhibition of hemolysis: thus, ++ indicates absolute inhibition, + indicates at least 50 per cent of inhibition, and +− any well marked degree of inhibition below 50 per cent. A diagnosis of lues should not be based upon a +− reaction unless there is a clear clinical history of infection or the patient is on specific treatment.

The 2,435 cases were divided as follows: Group 1, 1,661 cases; Group 2, 618 cases; and Group 3, 156.

GROUP 1: *Cases in which the diagnosis was lues or suspected lues, etc.*—Of this group of cases 1,661 were examined, of which 1,315, or

79.1 per cent, gave positive results. The cases were divided as regards the stage of the disease as shown in Table 1.

TABLE 1.

Results of the Complement Fixation Test in 1,661 Cases of Lues.

Stage of the Disease	No. of Cases	Positive	Negative	Percentage Positive
Primary	293	219	74	74.7
Secondary	723	658	65	91.
Tertiary	217	185	32	85.2
Latent	296	228	168	57.5
Congenital	19	17	2	89.4
Parasyphilitic	13	8	5	62.3
Totals	1,661	1,315	346	79.1

However, the above table is not a fair statement of the results actually obtained with the complement fixation test in our cases for there were 53 individuals tested in the primary stage of the disease at a period too early for the reaction to have appeared, while in 37 of the secondary cases giving a negative result vigorous specific treatment had been administered for weeks or months. Deducting these cases from the totals given in Table 1, we have 1,571 cases in Group 1, of which 1,315, or 83.7 per cent, gave a positive result. The cases were divided regarding the stage of the disease as shown in Table 2.

TABLE 2.

Results of the Complement Fixation Test after Deducting Cases Mentioned in Text.

Stage of Disease	No. of Cases	Positive	Negative	Percentage Positive
Primary	240	219	21	91.2
Secondary	686	658	28	95.9
Tertiary	217	185	32	85.2
Latent	396	228	168	57.5
Congenital	19	17	2	89.4
Parasyphilitic	13	8	5	62.3
Totals	1,571	1,315	256	83.7

Before considering the results in the various stages a little more in detail it is interesting to compare the percentage of positive results in these 1,571 cases of lues with the percentage of positive results obtained in the 361 cases of lues reported in the previous paper alluded to, as such comparison demonstrates the influence of numbers upon the percentage of positive results. In making this comparison the 13 parasyphilitic cases are deducted from the

1,571 cases, as this class was not included in the previous report. Table 3 gives the result in both series in the various stages of the disease.

TABLE 3.

COMPARISON OF THE RESULT OF THE COMPLEMENT FIXATION TEST IN SERIES 1 AND 2.

Series 1: 361 Cases			Series 2: 1,558 Cases		
Stage of Disease	No. Cases	Percentage Positive	Stage of Disease	No. Cases	Percentage Positive
Primary	76	85.5	Primary	240	91.9
Secondary	147	97.2	Secondary	686	95.9
Tertiary	74	82.	Tertiary	217	85.2
Latent	55	72.	Latent	396	57.5
Congenital	9	88.8	Congenital	19	89.4
Totals	361	87.7	Totals	1,558	83.8

It is evident from this table that the percentage of positive results in the primary, tertiary, and congenital cases increases with the number of individuals examined and that the percentage decreases slightly in the secondary stage and considerably in the latent stage. The decrease in the percentage of positive results in both these stages was due to the fact that a larger number of cases having had some specific treatment were tested, and such factors should always be considered in comparing the results of various observers with the complement fixation test.

Results in the primary stage.—All cases have been considered as primary which have presented the initial lesion and which were tested before the development of secondary symptoms. Of this class of cases 293 were examined, of which 219, or 74.7 per cent, were positive. These figures, however, include 53 individuals who were tested within two to four days after the appearance of the initial lesion, too early for the reaction to appear, and deducting these we have 240 cases, of which 219, or 91.2 per cent, were positive. The percentage of positive results in primary lues has varied from 38.6 per cent to 100 per cent, as given by various observers, and our results agree with those of the majority of observers who have used both the original Wassermann technic and the Noguchi modification. Some cases have never given a reaction in the primary stage, the reaction only appearing after the development of secondary symptoms.

Time of the appearance of the reaction.—In 140 of our cases we have been able to determine the date of the appearance of the positive reaction, and Table 4 gives the data in these cases. There have been a large number of cases which were tested within a month to six weeks after the appearance of the initial lesion, but the cases here recorded are the only ones in which we can be sure, within approximate limits, of the date of the appearance of a positive reaction. The intensity of the reaction is also indicated in the table.

TABLE 4.

SHOWING DATE OF APPEARANCE OF REACTION IN 140 CASES OF LUES.

Time after Initial Lesion	No. of Cases	Character of Reaction			Time after Initial Lesion	No. of Cases	Character of Reaction		
		++	+	+−			++	+	+−
5 days	6	2	3	1	17 days	3	3	..	..
6 days	1	..	...	1	18 days	2	2	..	..
7 days	4	3	..	1	19 days	2	2	..	..
8 days	4	2	2	..	3 weeks	34	24	4	6
10 days	3	1	1	1	1 month	25	18	5	2
11 days	7	4	3	..	5 weeks	7	2	5	..
13 days	4	3	1	..	6 weeks	16	9	5	2
2 weeks	8	6	1	1	7 weeks	2	1	1	..
16 days	2	1	1	..	2 months	10	6	4	..

An extended discussion of this table is not necessary as it is self-explanatory. It will be noticed that the date of the appearance of the first positive reaction varied all the way from five days to two months, the greater number of cases becoming positive in the third and fourth week after the appearance of the initial lesion. No less than six cases became positive in five days, while 10 did not become positive until two months had elapsed, and this well illustrates the great variation in the time of appearance of the reaction.

Results in the secondary stage.—There were 723 individuals examined during the secondary stage of lues, of whom 658, or 91 per cent, gave a positive result. From these should be deducted 37 individuals who had received vigorous specific treatment for weeks or months and who were receiving treatment at the time the test was made. Deducting these cases we tested 686 individuals in the secondary stage of lues, of whom 658, or 95.9 per cent, gave a positive result. It is evident that positive results are more frequent in the secondary than in the primary stage of lues, and

our percentage of positive results agrees with that of the majority of observers. There have been a few cases presenting well marked secondary lesions in which a positive result was not obtained, although repeated tests were made, and I think it must be admitted that we occasionally encounter a patient presenting severe primary, secondary, or even tertiary lesions in whom the Wassermann reaction remains negative.

Results in the tertiary stage.—There were 217 individuals examined in this stage of the disease. Of these 185, or 85.2 per cent, gave a positive reaction. Many of the patients examined had had more or less specific treatment, but these cannot be eliminated as so few would be left that the figures would have little value. Great variations in the results obtained by different observers with the complement fixation test have been noted in this stage of the disease, but they have depended largely upon the class of cases examined, as some authorities have included cases presenting no tertiary symptoms in this class, while others have only considered those in which marked symptoms were present. In nearly all of our cases some symptom of the tertiary stage was present at the time of the examination.

Results in latent lues.—In this class of cases are included all those in which no active symptoms of lues were present at the time of the examination but in which a clear history of infection was obtained. Almost all of these cases had received more or less specific treatment and the majority were tested in order to determine whether the disease had been cured. Of this class, 296 individuals were examined, of whom 228, or 57.5 per cent, gave a positive result. In most of these cases the only evidence which existed of lues was general glandular enlargement and in many even this symptom was absent. Our results are slightly higher in this class of cases than those given by the majority of observers, but the difference is slight, and indicates that in latent lues the technic recommended by Noguchi gives better results than that used in the original Wassermann test.

Congenital lues.—Only 19 cases of congenital lues have been tested, of which 17, or 89.4 per cent, gave a positive result. Our results are in agreement with those of the majority of observers,

using either the original Wassermann technic or the Noguchi modification.

Results in parasyphilitic conditions.—Ten patients suffering from tabes and three from paresis were tested, the total 13 giving eight positive results, or 62.3 per cent. All the paretics gave a positive result and five, or 50 per cent, of the patients suffering from tabes. Here again our results agree with those of observers who have used the original Wassermann technic.

GROUP 2: This group includes all cases in which the patient was suffering from some other disease than lues. Of this group 618 individuals were examined, of whom seven, or one per cent, were positive. Three of the positive results were obtained in patients suffering from tertian malarial fever, the blood being tested during the febrile stage, and in all the blood became negative after the subsidence of the fever. In the other cases, four in number, three were diagnosed as pulmonary tuberculosis, and one as "undetermined fever." The three cases diagnosed as tuberculosis gave strong reactions and recovered under specific treatment. In two of these a history of luetic infection was afterward obtained, while in the other such an infection could not be excluded. In the case diagnosed as "undetermined fever" the exact nature of the disease could not be learned, but it was probably malarial fever.

GROUP 3: The blood of 156 individuals in good health and in whom luetic infection could be excluded was tested and a negative result was obtained in every instance.

The specificity of the test.—As a result of our experience I believe that the complement fixation test is specific for lues if such conditions as leprosy, malarial fever, scarlet fever, and frambesia can be excluded. It is unnecessary to discuss in detail the results which have been obtained with the test in these diseases, but it is well known that a certain percentage of patients suffering from them give a positive reaction whether the original Wassermann technic be used in making the test or the Noguchi modification. A few other conditions have been observed in which positive results were obtained in isolated cases, as carcinoma, tuberculosis, and sepsis, but the cases reported are infinitesimal in number, and do not vitiate the practical value of this test in the diagnosis of lues, as a

latent luetic infection could not be excluded. I believe that a large percentage of positive results in non-luetic cases is proof of imperfect technic and that such reports must be viewed with suspicion.

If the diseases in which the complement fixation test has occasionally been found positive can be excluded, a double-plus or plus reaction is sufficient to enable one to diagnose the presence of lues. I am convinced that, under such conditions, the test is absolutely specific whether symptoms of the disease are present or not, and whether there is, or is not, a history of infection. In those cases in which, after the appearance of a suspicious lesion, the negative reaction becomes positive, a diagnosis of lues can be made without hesitation. On the other hand, a diagnosis of lues should never be made upon a plus-minus reaction alone.

The value of a negative reaction is not as great as that of a positive one. A considerable proportion of cases of lues do not give a positive reaction, even though symptoms are present, and for this reason the disease cannot be excluded on the strength of a negative result. In the interpretation of a negative result the history of the case, the symptoms present, and the amount of previous specific treatment must all be carefully considered.

The test as a control of treatment.—In a very large number of our cases the test has been used as a control of treatment both with mercury and with arsenobenzol (Salvarsan). Our results demonstrate the very great value of the test as an index of the efficacy of treatment, especially of treatment with arsenobenzol. At the present writing tests are being made upon over 700 patients who have been treated with this drug, the blood being tested every month in the majority of the cases. We have been able to trace the gradual disappearance of the reaction in treated cases and in a certain proportion its gradual reappearance before a clinical relapse occurred. The data derived from the test has made possible the intelligent use of this drug and of mercury, in the treatment of both initial infections and relapses.

The test has also demonstrated that even prolonged treatment with mercurials and potassium iodide does not cause the reaction to disappear permanently, although in many cases treatment with

mercury, if the drug is being taken at the time of the examination, will render the test negative or only weakly positive. In our experience mercury should always be omitted for at least three to four weeks before the blood is tested, as otherwise negative results will often be obtained in positive cases.

In order to show the persistence of positive reactions after mercurial treatment I have compiled the results in a number of our cases and they are given in Table 5.

TABLE 5.

ILLUSTRATING THE PERSISTENCE OF THE COMPLEMENT FIXATION TEST AFTER MERCURIAL TREATMENT.

Method of Mercurial Treatment	Length of Treatment	No. of Cases	Intensity of the Reaction		
			++	+	+−
Internal	1 month	2	..	2	..
"	2 months	3	1	2	..
"	3 "	4	2	2	..
"	5 "	4	1	3	..
"	6 "	7	4	1	2
"	7 "	3	2	1	..
"	8 "	3	3	..	..
"	9 "	10	7	1	2
"	10 "	2	1	1	..
"	11 "	3	3	..	..
"	1 year	19	9	5	5
"	14 months	2	2	..	..
"	15 "	2	1	1	..
"	16 "	4	3	..	1
"	17 "	1	1	..	..
"	18 "	7	1	4	2
"	19 "	2	2	..	..
"	20 "	1	..	1	..
"	2 years	15	8	5	2
"	2.5 "	3	..	2	1
"	3 "	5	1	2	2
"	4 "	1	1	..	..
Internal*	7 "	1	..	1	..
Internal*	10 "	1	1	..	..
Internal*	12 "	1	..	1	..
Inunctions	2 months	2	2	..	..
"	5 "	1	..	1	..
"	6 "	2	2	..	..
"	1 year	1	1	..	..
Injections	4 injections	1	1	..	..
"	6 "	1	1	..	..
"	14 "	1	1	..	..
"	16 "	2	..	2	..
"	25 "	2	..	2	..
Injections and Inunctions	15 months	1	..	1	..
" " "	7 "	1	..	1	..
" " "	8 "	1	1	..	..

* Interrupted treatment during this time.

The consideration of this table makes evident the fact that a large number of patients upon internal treatment with mercury show a positive reaction after months or even years of such treatment, and that even treatment for a long period by injections or

inunctions does not prevent the occurrence of a positive reaction in many instances.

The use of the test as a control of treatment has demonstrated the much greater specific value of arsenobenzol than of mercury. In many instances, the complement fixation reaction has been found positive after months or even years of mercurial treatment, but has quickly disappeared after the administration of arsenobenzol. I have selected from our data the most interesting cases of this kind and have included them in Table 6.

TABLE 6.

SHOWING THE EFFECT OF TREATMENT WITH ARSENOBENZOL UPON THE COMPLEMENT FIXATION TEST IN CASES SHOWING A POSITIVE REACTION AFTER MERCURIAL TREATMENT.

No.	Method of Mercurial Treatment	Length of Mercurial Treatment	Reaction	Dose of Arsenobenzol	Time of Disappearance of Reaction after Arsenobenzol
1.....	Internal	7 months	+	0.5 gm. intrav.	6 weeks
2.....	"	7 "	+	0.6 gm. intramus.	3 "
3.....	Injections and Internal	7 "	+	0.6 " "	9 "
4.....	Internal	7 "	++	0.6 " "	5 "
5.....	"	8 "	+	0.2 gm. intrav. 0.6 gm. intramus.	6 "
6.....	"	9 "	+	0.6 gm. intrav. 0.6 gm. intramus.	3 "
7.....	"	9 "	++	0.6 " "	6 "
8.....	"	1 year	++	0.6 " "	5 "
9.....	Inunctions and Injections	1 "	+	0.6 " "	8 "
10.....	Internal	1 "	++	0.6 " "	2 "
11.....	Injections and Internal	1 "	++	0.5 gm. intramus. 0.5 gm. intrav.	5 "
12.....	Internal	1 "	++	0.5 gm. intramus.	3 "
13.....	"	1 "	++	0.6 " "	5 "
14.....	"	1 "	++	0.6 " "	3 "
15.....	"	13 months	+	0.6 " "	7 "
16.....	"	18 "	++	0.6 " "	2 "
17.....	"	2 years	++	0.6 " "	8 "
18.....	Internal and Injections	2 "	+	0.6 " "	2 "
19.....	Internal	3 "	+	0.6 " "	3 "
20.....	Internal and 25 Injections	3 "	++	0.5 " "	7 "

It is evident from this table that arsenobenzol causes the complement fixation reaction to disappear in cases which have retained it under mercurial treatment for long periods of time, and when one considers that these cases were all resistant clinically to mercurial treatment the prompt disappearance of the reaction under arsenobenzol is remarkable.

Perhaps the best illustration of the ease with which one can use the test as a control of treatment is shown in experiments upon infected rabbits. Through the kindness of Captain Nichols, of the Army Medical Corps, I have been able to test the serum of

rabbits infected with both lues and yaws, both before and after treatment with arsenobenzol. Table 7 shows the results obtained before and after treatment in a rabbit infected with lues, and Table 8 the results in a rabbit infected with yaws.

TABLE 7.

RESULTS OF COMPLEMENT FIXATION TEST BEFORE AND AFTER TREATMENT WITH ARSENOBENZOL IN RABBIT NO. 39, INFECTED WITH LUES.

Amount of Serum	Date of Tests								Remarks
	Jan. 23	27	30	Feb. 6	8	10	13	18	
0.05 c.c.	−	+	+	++	−	+	o	−	Spirochaetes found in testicular lesion Jan. 19, 1911
0.10 c.c.	−	++	++	++	+−	++	−	−	
0.15 c.c.	o	++	++	++	+−	++	o	−	0.02 gm. arsenobenzol per kilo administered intravenously Feb. 6, 1911
0.20 c.c.	++	o	++	++	+−	++	−	−	

o = no test.

TABLE 8.

RESULTS OF COMPLEMENT FIXATION TESTS BEFORE AND AFTER TREATMENT WITH ARSENOBENZOL IN RABBIT NO. 44, INFECTED WITH YAWS.

Amount of Serum	Date of Tests								Remarks
	Jan. 23	30	Feb. 2	6	8	10	13	17	
0.05 c.c.	−	−	++	++	−	−	−	−	Spirochaetes found in lesion Jan. 21, 1911
0.10 c.c.	−	+−	++	++	−	+−	−	−	0.02 gm. arsenobenzol per kilo administered intravenously Feb. 6, 1911
0.15 c.c.	−	+	++	++	+−	+	−	−	
0.20 c.c.	−	++	++	++	+−	++	−	−	

These tables illustrate how accurately one may follow the effect of treatment upon the complement fixation reaction, and in scores of our human cases we have been able to trace, week by week, the gradual disappearance of the reaction.

A negative reaction following treatment, unless it be repeatedly so, cannot be regarded as an indication that the infection is cured, for to be of value the test should be repeated at intervals of a month or two, for at least a year. If after thorough treatment the test remains negative for that length of time, I believe that it is justifiable to conclude that the infection is cured, provided the patient originally gave a positive reaction. *The presence of a reaction indicates active infection even though it may not be manifested by symptoms, and is always an indication that further treatment is necessary.* A

negative result in the early period of the disease is of little value, but becomes more and more so the later the stage of the disease, the longer the duration of treatment, and the longer the reaction has remained negative. Many cases become negative after a short course of mercurial treatment, but practically all of these will give a positive reaction if the treatment be omitted for from three weeks to one month. It cannot be too strongly emphasized that a single negative reaction after treatment is of no value as an indication of cure except in patients who have been very thoroughly treated, and who have been free from symptoms for a year or more, and even in such cases it is best to have the test repeated at the end of another six months or a year.

Factors influencing the result of the complement fixation test.—I have already spoken of the influence of certain other diseases and of previous specific treatment upon the result of the complement fixation test, and desire, in conclusion, to call attention to certain other phenomena which have been observed in our routine application of the test and which are of both scientific and practical interest.

The relation of certain bacteria to the results of the test.—In recent experiments I have demonstrated[1] that certain strains of staphylococci and streptococci, when growing in normal serum, under favorable conditions, are capable of producing certain substances in the serum which cause a positive result with the complement fixation test for lues. In order that this may occur the bacteria must develop in the serum at incubator temperature for at least 24 hours or at room temperature for from five to seven days. In addition, every strain of a certain bacterial species will not produce this result as was shown by the fact that while a double-plus reaction occurred in normal serum infected with a stock *Staphylococcus aureus* and with a *Staphylococcus aureus* isolated from a normal serum giving a positive reaction, negative results were obtained with another *Staphylococcus aureus* isolated from a serum giving a negative reaction.

These non-specific reactions do not occur in normal sera which are sterile, even though the sera be kept at room temperature for

[1] *Jour. Exp. Med.*, 1911, 13, p. 521.

as long a period as one month. Such reactions are in all probability very rare in practice, but the fact that they can occur as a result of bacterial activity is of enough importance to justify the use of aseptic methods in the collection of blood for the Wassermann test. In two sera which gave a negative reaction on the first test, infection with a streptococcus caused the reaction to become positive a week later, and in the only instance we have observed in which a presumably normal serum, on the first test, gave a positive reaction, bacterial contamination was probably the cause.

The effect of the ingestion of alcohol upon the results of the test.—Working in conjunction with Captain Nichols, of the Army Medical Corps, it has been demonstrated[1] that the ingestion of considerable amounts of alcohol will render a positive serum negative if it be tested within 24 hours after alcohol has been administered, and in some cases the serum will give a negative reaction for three days after the administration of this drug. We tested nine individuals, all of whom gave a double-plus complement fixation test, administering quantities of alcohol varying from 90 c.c. to 120 c.c. in the form of beer and whiskey, and found that in every case the positive reaction disappeared within 24 hours after the administration of the drug, and that in one case it did not become positive again until the end of three days, while in the others the serum again became positive within 36 hours.

These observations have demonstrated that no dependence can be placed on a negative Wassermann reaction in individuals who have, within 24 hours of the collection of the blood, ingested a considerable amount of alcohol, while in some instances the drug may render the reaction negative for as long a period as three days. All of our tests were made upon individuals giving absolute inhibition of hemolysis and it is probable that the drug will be found to have a still more decisive effect upon weaker reactions, even though given in much smaller quantities. Experiments are now under way to determine the smallest amount of alcohol which will render the test negative and the effect of the drug upon cases giving a plus or a plus-minus reaction. In view of the effect of alcohol upon the complement fixation test a careful inquiry should always

[1] *Jour. Am. M. Ass.*, 1911, 52, p. 474.

be made regarding the recent use of this drug before collecting blood for the complement fixation test.

In the military service the complement fixation test has proved of the greatest value in the diagnosis of obscure and latent infections; in preventing the enlistment of luetic individuals; and in controlling treatment both with mercurials and arsenobenzol. The test is being used as a routine measure in the laboratories of the Medical Department of the army both in this country and the Philippine Islands, and continued use has only increased our confidence in its value as a diagnostic measure.

THE EFFECT OF SPECIFIC VACCINES ON RAT TYPHOID.*†

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It is impossible to produce typhoid fever in small laboratory animals by feeding them with typhoid bacilli; therefore the immunity produced by specific vaccines has always been tested by subcutaneous or intraperitoneal inoculations of the living culture. These methods, however, do not reproduce a disease at all comparable to human typhoid.

Rats and mice, on the contrary, when fed with certain of the paratyphoid group contract a disease which closely resembles typhoid in man. This fact has been taken advantage of by a number of workers for a comparative study of the specific vaccines. Chief among these investigators are Loeffler, Marks, and recently Bruckner.

Loeffler,[1] working with his culture of *B. typhimurium* on field mice, found that all subcutaneous inoculations failed to protect against subsequent feeding, but in a few instances he apparently secured protection from previous feeding with dead or living cultures.

Marks,[2] working with the same culture on mice, was however unable to immunize his animals against subsequent feeding. More recently Bruckner[3] has tested the local and general immunity in white mice to paratyphoid bacillus *B*. He was able by continued previous feeding of small doses of living cultures to protect them against subsequent subcutaneous injections.

Metchnikoff and Besredka[4] have just published their work on

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† We wish to thank Dr. Park, director of the Research Laboratory, Board of Health of New York, for his courtesy in permitting us to complete the work in his laboratory.

[1] *Festschrift*, Bd. 1.

[2] *Arb. aus dem König. Inst. f. Exp. Therap.*, Frankfort, 1908, 4, p. 37.

[3] *Ztschr. f. Immunitäts.*, 1 Orig., 1911, 8, p. 434.

[4] *Ann. de l'Inst. Pasteur*, 1911, 25, p. 193.

typhoid in chimpanzees and gibbons. They were able to reproduce the disease in these animals by feeding them living cultures of human typhoid; but specific vaccines and previous feeding with dead bacilli failed completely to protect their animals from oral infection. They stated that the immunity produced by the subcutaneous or intraperitoneal inoculations of the specific vaccines is local, and has no effect on the infecting organisms that gain entrance to the body through the intestinal wall, reaching conclusions directly opposed to those of Bruckner, Calmette, and others.

Our experiments, which have been carried on for the last two years, were begun on white mice, using the commercial Danysz virus, one of the Gaertner group, as the test organism. Cultures killed by heat, Vaughan's residue, and protective inoculations of immune serum were tested. The feeding of dead bacilli caused death in mice, as the Danysz bacillus has a thermostabile endotoxin. All the injections were given subcutaneously, to make them more comparable with the method of inoculation of human beings. When later the vaccinated mice were fed with living cultures, no protection was shown; sickness, usually fatal, occurred exactly as in the untreated mice.

TABLE 1.

MICE INOCULATED SUBCUTANEOUSLY OR FED WITH DEAD BACILLI LATER TESTED AGAINST FEEDING WITH LIVE CULTURES.

White Mouse	Previous Treatment	Doses	Number of Days' Interval before Feeding with Live Culture	Treatment	Result
No. 1	None	None	None	....	Sick—died in 3 days
No. 2	None	None	None	....	Sick—died in 7 days
No. 3	None	None	None	....	Sick—recovered
No. 4	Culture killed by heat	1–200 cult. subcut.	6 days	....	Sick—died in 5 days
No. 5	Culture killed by heat	Ditto	6 days	....	Sick—died in 10 days
No. 6	Culture killed by heat	Ditto	6 days	....	Sick—recovered
No. 7	Immune serum (rabbit)	½ c.c. ser. subcut.	None	2 days later ½ c.c. ser. subcut.	Sick—died in 23 days
No. 8	Ditto	Ditto	None	Ditto	Sick—recovered
No. 9	Ditto	Ditto	None	Ditto	Sick—recovered
No. 10	Vaughan's residue	2.5 mg. subcut.	6 days	....	Sick—died in 3 days
No. 11	Vaughan's residue	2.5 mg. subcut.	6 days	....	Sick—died in 4 days
No. 12	Vaughan's residue	2.5 mg. subcut.	6 days	....	Sick—died in 15 days
No. 13	Vaughan's residue	2.5 mg. subcut. two doses at 4 days' interval	2 days	....	Sick—died in 9 days
No. 14	Vaughan's residue	Ditto	2 days	....	Sick—died in 15 days

Results.—Previous vaccination with cultures killed by heat or Vaughan's residue failed to protect mice against subsequent feeding of live cultures. There was an apparent protection with immune serum but this was not borne out on repeating the experiments as shown in Table 2. All the vaccinations were repeated, giving the same results.

TABLE 2.

Mice Resistant from Table 1, Re-fed Live Cultures—Also Serum and Vaccine Tests Repeated.

Mouse	Previous Treatment	Feeding of Live Cultures	Number of Days' Interval before Feeding with Live Culture	Result
No. 1.....	None	None	None	Sick—died in 2 days
No. 2.....	None	None	None	Sick—died in 7 days
No. 3.....	None	None	None	Sick—recovered
No. 4.....	None	Once—sick, recovered	45 days	Sick—died in 6 days
No. 5.....	Immune serum 2 doses	Once—sick, recovered	45 days	Sick—died in 9 days
No. 6.....	Immune serum 2 doses	Once—sick, recovered	45 days	Sick—died in 10 days
No. 7.....	Culture killed by heat 6 days later	Once—sick, recovered	45 days	Sick—died in 9 days
No. 8.....	Vaughan's residue—2.5 mg.	None	4 days	Sick—died in 2 days
No. 9.....	Ditto	None	4 days	Sick—died in 9 days
No. 10.....	Killed cultures 1 dose	None	4 days	Sick—died in 6 days
No. 11.....	Immune serum ¼ c.c. subcut.	None	None	Sick—died in 7 days
No. 12.....	Ditto	None	None	Sick—died in 9 days
No. 13.....	Ditto	None	None	Sick—died in 11 days

Note.—The feeding of mice with dead bacilli caused their death, also subcutaneous inoculations of dead bacilli were often toxic. Vaughan's residue inoculated subcutaneously failed to protect mice from later intraperitoneal inoculations of the bacilli, although it did protect guinea-pigs thus treated from similar injections.

Method.—An emulsion was made from agar cultures of the Danysz bacillus. Small cubes of stale bread were moistened with 1 c.c. each of this emulsion. The mice, which had been kept without food for 12 to 24 hours, were separated, each receiving one cube of the infected bread. The next day after each one had eaten the bread, the mice were returned to a common receptacle. Autopsies showed congestion of the spleen, liver, and of the intestinal mucous membrane, and usually enlargement of Peyer's patches. Cultures were recovered from spleen and heart's blood in most cases. The feeding of all the treated and untreated mice was done at the same time, with the same emulsion, so that each

set acted as a standard of comparison for the others. The same method was used in all the following experiments except that the dose of the emulsion was increased for the rats.

After an interval, the mice that recovered were re-fed with the living culture and all contracted the disease again, showing that no immunity had been established even by a previous attack, thus differing from the results of Loeffler, who worked on field mice and with another organism; but agreeing with the results of Marks.

Results.—There was no apparent protection by the use of any specific method of vaccination; not even a previous attack of the disease gave rise to immunity in mice. The protection with the serum shown in Table 1 could not be reproduced, although the experiment was repeated a number of times. The individual resistance to the feeding plays a very important part as shown by the untreated mice, in which one died the second day after feeding and the other recovered, the only recovery in this series of 13.

Rats being less susceptible than mice, the experiments were repeated upon them. The vaccines used were cultures killed by heat, Vaughan's residue, sensitized bacilli (Besredka's vaccine), and preliminary feeding with small doses of the living culture. The technic was the same as that used for the mice. The animals injected subcutaneously with Vaughan's residue and those injected with the sensitized bacilli were not protected against later feedings of living cultures.

The rats injected with cultures killed by heat when fed later with living cultures all contracted the disease, but one-third recovered, whilst the disease was uniformly fatal for the rats vaccinated by other methods and the untreated controls.

The rats given preliminary small doses of living culture when fed later large doses of the living culture—the same dose that was uniformly fatal for the controls—were not even ill, complete protection having been established.

Results.—Previous vaccinations with sensitized bacilli did not protect against subsequent feeding with living culture. All the rats died, as did the controls, but in the rats vaccinated with cultures killed by heat, one-third were protected from death,

TABLE 3.

THE EFFECT OF FEEDING WITH LARGE DOSES OF LIVE CULTURE ON UNTREATED AND TREATED RATS.

Rat	Previous Treatment	Interval before Feeding with Live Culture	Result
No. 1. White control	None	None	Sick—died 6 days
No. 2. White control	None	None	Sick—died 6 days
No. 3. White control	None	None	Sick—died 27 days
No. 4. Gray and white control...	None	None	Sick—died 6 days
No. 5. Gray and white control ...	*Sensitized bacilli* (Besredka's vaccine) 1/5 c.c. cult. subcut.	40 days	Sick—died 6 days
No. 6. Gray and white control....	Ditto	40 days	Sick—died 6 days
No. 7. Gray and white control ...	Ditto	40 days	Sick—died 9 days
No. 8. White control	*Sensitized bacilli* (Besredka's vaccine) 1/100 c.c. cult. subcut.	40 days	Sick—died 6 days
No. 9. White control	Ditto	40 days	Sick—died 9 days
No. 10. White control	Ditto	40 days	Sick—died 12 days
No. 11. White control	*Culture killed by heat* 1/1000 agar cult. subcut., 7 days later 1/1000 agar cult. subcut.	41 days	Sick—died 6 days
No. 12. White control	Ditto	41 days	Sick—died 10 days
No. 13. White control	Ditto	41 days	Sick—recovered
No. 14. White control	*Culture killed by heat* 1/1000 agar cult. subcut., 7 days later 1/100 agar cult. subcut.	41 days	Sick—died 6 days
No. 15. White control	Ditto	41 days	Sick—died 6 days
No. 16. Black and white..........	Ditto	41 days	Sick—recovered
No. 17. Gray..	*Small doses live cult.* 1/2 agar cult.—not sick	22 days	Not sick
No. 18. Gray........	Ditto	22 days	Not sick
No. 19. Gray........	*Small doses live cult.* 1 agar cult.—not sick	22 days	Not sick
No. 20. Gray........	Ditto	22 days	Not sick
No. 21. White.......	*Small doses live cult.* 1/10 agar cult.—not sick	22 days	Not sick
No. 22. White.......	Ditto	22 days	Not sick
No. 23. White.......	*Small doses live cult.* 2/5 agar cult.—not sick	22 days	Not sick
No. 24. White.......	*Small doses live cult.* 2/5 agar cult.—died in 10 days		
No. 25. White.......	*Small doses live cult.* 1/10 agar cult.—not sick	69 days	Not sick
No. 26. White.......	Ditto	69 days	Not sick
No. 27. White.......	Ditto	69 days	Not sick
No. 28. White.......	Ditto	69 days	Not sick
No. 29. White.......	Vaughan's residue 3 subcut. inoculations at 4 days' interval, followed in 33 days by feeding of 1/10 agar cult.	69 days	Not sick
No. 30. White.......	Ditto	69 days	Not sick
No. 31. White.......	Ditto	69 days	Not sick
No. 32. White.......	Ditto	69 days	Not sick
No. 33. White.......	*Sensitized bacilli* 1/4 agar slant subcut., 14 days later 1/6 agar slant subcut., 7 days later fed 1/10 live culture—not sick	69 days	Not sick
No. 34. White.......	Ditto	69 days	Not sick
No. 35. White	Ditto	69 days	Not sick
No. 36. White.......	Ditto	69 days	Not sick
No. 37. White.......	*Culture killed by heat* 1/10 c.c. killed culture (5,000,000) subcut., 4 days later 2/10 c.c. killed culture subcut., 6 days later 4/10 c.c. killed culture subcut., 27 days later fed 1/10 live culture—not sick	69 days	Not sick
No. 38. White.......	Ditto	69 days	Not sick
No. 39. White.......	Ditto	69 days	Not sick

although all contracted the disease. The best protection, however, was afforded by small preliminary feedings of living cultures, as in the rats thus treated none showed any signs of sickness on subsequent feeding.

The rats which had resisted the feeding with large doses of the living culture, after an interval were tested as to their immunity against intraperitoneal inoculations. Although the results were not uniform, usually there was an increased resistance established, which either caused a delayed death, or, in a few cases, recovery. This increased resistance was as great in those rats which had only been fed several times with living cultures as in those which had been previously vaccinated and then fed with small doses of living cultures followed later by larger ones. The rats vaccinated subcutaneously did not show any greater intraperitoneal resistance than those treated by mouth.

TABLE 4.

THE EFFECT OF INTRAPERITONEAL INOCULATIONS ON RATS IMMUNE TO FEEDING OF LIVE CULTURES.

Rat	Previous Treatment	Number of Days Interval	Intraperitoneal Inoculation Dose	Result
No. 1. White..	None	None	$\frac{1}{2}$ agar culture	Died in 2 days
No. 2. White..	1 dose sensitized bacilli subcut. 14 days later repeated, 7 days later fed small dose live culture, 69 days later fed large dose live cult. — not sick (controls died)	30 days	$\frac{1}{2}$ agar culture	Died in 4 days
No. 3. White..	Fed small doses live culture—not sick, 69 days later fed large dose cultures —no sickness developed (controls died)	30 days	$\frac{1}{2}$ agar culture	Died in 8 days
No. 4. White..	None	None	$\frac{1}{10}$ agar culture	Died in 7 days
No. 5. White..	The same as for Rat No. 2	30 days	$\frac{1}{10}$ agar culture	Sick, recovered
No. 6. White..	The same as for Rat No. 3	30 days	$\frac{1}{10}$ agar culture	Sick, recovered
No. 7. White..	None	None	$\frac{1}{100}$ agar culture	Died in 7 days
No. 8. White..	The same as for Rat No. 2	30 days	$\frac{1}{100}$ agar culture	Sick, recovered

Result.—All the rats previously fed with live culture, whether vaccinated subcutaneously or not, showed an increased resistance to subsequent intraperitoneal inoculations, the subcutaneous vaccinations not apparently increasing this resistance.

Note.—The growth of culture on agar slant was more abundant than in Table 4, as a $\frac{1}{2}$ agar culture killed here in 18 hours, while in Table 4 it took two days.

Result.—As in Table 4 a resistance to intraperitoneal injection was shown to multiple lethal doses, except for one rat, which,

although immune to the feeding of living cultures, showed no increased resistance to intraperitoneal inoculations.

TABLE 5.

TESTING OF INTRAPERITONEAL RESISTANCE TO MULTIPLE LETHAL DOSES IN RATS IMMUNE TO FEEDING OF LIVE CULTURES.

Rat	Previous Treatment	Number of Days' Interval	Intraperitoneal Inoculation	Result
No. 1.........	None	None	$\frac{1}{2}$ agar culture	Died in 18 hrs.
No. 2.........	3 subcut. inoc. of Vaughan's residue (5 mg.) at 4 days' interval, 33 days after last inoculation fed small dose of live culture—not sick (controls not sick), 69 days after feeding fed large dose culture—not sick. (controls died)	35	$\frac{1}{2}$ agar culture	Died in 4 days
No. 3.........	$\frac{1}{10}$ c.c. killed culture subcut. inject. (ca. 5,000,000) bact., 4 days later $\frac{2}{10}$ of killed culture, 6 days later $\frac{4}{10}$ of killed culture, 27 days after last injection fed small dose live culture—not sick (controls not sick), 69 days after this feeding fed a large dose culture—not sick (controls died)	35	$\frac{1}{2}$ agar culture	Died in 4 days
No. 4.........	Fed small dose of live culture—slightly sick, 22 days later fed large dose live culture—not sick (controls died)	35	$\frac{1}{2}$ agar culture	Died in 18 hrs.
No. 5.........	None	None	1 agar culture	Died in 18 hrs.
No. 6.........	The same as for Rat No. 2	35	1 agar cultur	Died in 3 days
No. 7.........	The same as for Rat No. 3	35	1 agar culturee	Died in 5 days
No. 8.........	The same as for Rat No. 4	35	1 agar culture	Sick, recovered
No. 9.........	The same as for Rat No. 2	35	2 agar cultures	Died in 18 hrs.
No. 10........	The same as for Rat No. 3	35	2 agar cultures	Sick, recovered

Results.—In white mice the vaccination with cultures killed by heat, with Vaughan's residue, the treatment with immune serum, or previous feeding with live cultures failed to protect from subsequent feeding of large doses of living cultures.

In rats vaccination with sensitized bacilli gave no protection against subsequent feeding of large doses of living cultures.

Vaccination with cultures killed by heat saved one-third of the animals from death.

Preliminary feeding with small doses of living cultures gave complete protection against subsequent feeding of large doses of living cultures—doses which were uniformly fatal to the untreated control animals.

Conclusions.—In this work our results vary by varying the experimental animals. In all questions of immunity we think not alone the method of producing immunity but also the species and even

the individuals form a great factor, as well as the organism being tested.

From our results on rats we believe that, at least for them, the immunity produced by feeding is a general and not a local one, agreeing with Calmette, Bruckner, and others.

Thus far, we have been unable to produce a complete immunity against oral infection by subcutaneous inoculations, but the results with the cultures killed by heat were encouraging.

Further experiments with rats on vaccination, feeding with dead bacilli, specificity of the immunity, and treatment, specific and otherwise, are now being carried on, and the results will be given later.

SECONDARY INFECTION IN PULMONARY TUBERCULOSIS. THE RECOVERY OF THE STREPTOCOCCUS AND PNEUMOCOCCUS FROM THE BLOOD.*†

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The degree of importance of secondary infections in pulmonary tuberculosis is a much disputed question. A certain group of observers consider the tubercle bacillus as responsible for practically all the pathological conditions in tuberculosis of the lung, while others believe that the secondary invaders, more often streptococci, pneumococci, and staphylococci, play a very large rôle in the production of the changes. Between these two extremes stand those who assign a certain import to both the primary and the secondary invaders: some holding the opinion that the major rôle is played by the tubercle bacillus, others that the tubercle bacillus is of lesser importance and that the major rôle is played by the secondary invaders.

There is a large amount of evidence advanced in support of each of these views. This evidence, however, is for the most part incomplete and contradictory. The complexity of the problem is such that many of the methods which have been employed are of little value in furnishing reliable data, and in reviewing the literature one is impressed with the great variety of conclusions drawn, even by those using the same methods of investigation.

Seven methods of attack have been variously employed in the study of secondary infection in pulmonary tuberculosis, namely: (1) the direct observation of clinical phenomena, (2) animal experimentation, (3) bacteriological and anatomical examination of the lung after death, (4) bacteriological study of the sputum, (5) study of the opsonic index, (6) study of the leukocytes, and (7) blood cultures.

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The study of the clinical phenomena has led to direct contradictions. Baumler,[1] for instance, believes the frequent occurrence of bronchopneumonia after hemorrhage to be due to secondary infection, while Sorgo[2] contends that the tubercle bacillus itself is capable of producing the bronchitis or pneumonia.

Czaplewski, Ziegler, Maragliano, Weichselbaum, and Strumpel (all cited by Cornet[3]) on the basis of clinical studies, believe that secondary organisms are intimately concerned in the pathology of chronic pulmonary phthisis and, on the same basis, Cornet and Petruschky[4] are convinced that the steep temperature curve frequently seen in chronic pulmonary tuberculosis is due to a mixed infection. On the other hand, Pick[5] points out that this type of fever has been observed in cases showing only tubercle bacilli in the sputum and no secondary organisms in the tissues after death.

Rapid emaciation, excessive weakness, cough, profuse expectoration, chills, and sweats are all attributed to the presence of secondary pyogenic organisms; on the other hand, individuals having many secondary organisms in the sputum and pronounced cavities may not show fever, sweats, or emaciation (Sorgo).

Likewise the evidence based upon animal experiment is in a large measure contradictory. Prudden,[6] for instance, found that the inoculation of tubercle bacilli into rabbits produced tuberculosis, but seldom caused cavities; the intratracheal injection of streptococci into tuberculous animals, however, caused marked cavity formation. Marmorek,[7] on the other hand, was able to produce cavities by injecting pure cultures of tubercle bacilli together with large quantities of their toxins.

The evidence contributed by bacteriological and anatomical examination of the lung after death is to a large degree unreliable—postmortem, agonal, and terminal invasions being complicating factors of marked frequency and extent. However, secondary organisms have been described in the walls of cavities where during life they kept pace with the tubercle bacillus, or even preceded it in the invasion of healthy tissue (Cornet). Kossel and Cornet have both found secondary organisms in tubercles in the liver and spleen widely separated from the original seat of infection.

Sputum examination has been employed extensively in the study of secondary infections in pulmonary tuberculosis, but this method again must be recognized as having sharp limitations. It is a well-known fact that all organisms present in mixed infections in tuberculosis may also be found in the healthy mouth, pharynx, and trachea. Whether these organisms may be present normally in the alveoli of the lung is an open question.[8] Little more weight can be given to the results obtained by examination of washed tuberculous sputum, although the results of Kitasato,[9] Petruschky,[10] Spengler,[11] Schabad,[12] and Cornet agree in designating the streptococcus as the secondary organism most constantly recovered in such examinations. Sorgo maintains that the amount of washing used by these observers was not sufficient, and according to his extremely stringent rules mixed infection is much more rare than the above observers are led to believe.

[1] *Deut. med. Wchnschr.*, 1893, 19, p. 1.

[2] *Ztschr. f. klin. Med.*, 1907, 51, p. 250.

[3] "Tuberculosis and Acute General Miliary Tuberculosis" in Nothnagel's *Practice of Medicine*, W. B. Saunders & Co., Philadelphia, 1904, p. 583.

[4] *Ztschr. f. Hyg.*, 1894, 17, p. 59.

[5] *Wien. klin. Rundschau*, 1905, 19, p. 253.

[6] *New York Med. Jour.*, 1894, 60, p. 1.

[7] *Compt. rend. Soc. de Biol.*, 1904, 66, p. 60.

[8] *Jour. Exp. Med.*, 1905, 7, p. 78.

[9] *Ztschr. f. Hyg. u. Infektionskr.*, 1891, 11, p. 441.

[10] *Op. cit.*

[11] *Ztschr. f. Hyg. u. Infektionskr.*, 1894, 18, p. 343.

[12] *Ztschr. f. klin. Med.*, 1897, 33, p. 476.

The opsonic index has been employed by Wirths[1] to determine the existence of a mixed infection in tuberculosis and also the identity of the particular secondary invader. In a series of clinical cases Wirths found the index constantly normal, i.e., between 0.8 and 1.2, in all cases to the *Diplococcus capsulatus*, *Micrococcus tetragenus*, *Micrococcus catarrhalis*, meningococcus, pneumobacillus, pseudodiphtheria bacillus, colon bacillus, *B. subtilis*, but abnormal to the influenza bacillus in two cases of 17 (12 per cent), to the pneumococcus in 18 cases of 24 (75 per cent), to the streptococcus in six cases of 19 (30 per cent). He found the index to the four last organisms normal in five cases of 25 (20 per cent). The actual value of the opsonic index is yet to be determined by a more extensive application; at all events, the results are not at present uniform in the hands of all workers.[2]

Leukocytosis in pulmonary tuberculosis has been employed as a criterion indicative of a mixed infection (Simon)[3] inasmuch as the tubercle bacillus alone, except in acute miliary tuberculosis, does not produce a leukocytosis. Pick, Warthin, v. Jaksch, and Galbraith found the number of leukocytes in uncomplicated tuberculosis normal or low. Strauer, Grawitz, Halbron, and Appelbaum found the leukocytes normal in incipient cases. Ullon and Craig[4] also made leukocyte counts on a considerable number of cases and found an average of 10,285 in cases of the first stage; 12,772 in the second stage; and 14,041 in the third stage. I have made leukocyte counts on 112 cases and found an average of 11,963 in 16 incipient cases, 14,783 in 84 advanced cases, and 15,820 in 12 far-advanced cases.

A certain grade of leukocytosis is not, of course, rare in chronic pulmonary tuberculosis and a distinct leukocytosis is perhaps indicative of a mixed infection; the limitation of the method in the study of secondary invaders in tuberculosis is, however, that a secondary invader may be present without producing an evident leukocytosis, and furthermore, that the leukocytosis when occurring gives no information concerning the character or location of the secondary invading organism.

Finally there remains to be considered the blood-culture method of investigation. It has long been recognized that the finding of secondary invaders in the blood stream in pulmonary tuberculosis would constitute most direct evidence as to the importance of the rôle played by such organisms, and blood cultures have therefore been repeatedly employed in attempts to show the existence of such a bacteremia. As in the case of the other methods heretofore cited, however, directly contradictory deductions have been made by those employing this method. But in this instance I believe that the confusion in results is due to variations in the technic used rather than to the method itself; and from a study

[1] *Beiträge z. klin. d. Tuberk.*, 12, p. 159.

[2] I have determined the opsonic index to the streptococcus, pneumococcus, and staphylococcus in 40 cases of pulmonary tuberculosis and have found the index between 0.8 and 1.2 in all cases but one, and in this case the index to the *Staphylococcus aureus* was 0.75. As in Wirths' work, heterologous strains were used; possibly the result would have been different had homologous strains been employed.

[3] *Clinical Diagnosis*, Lea Bros. & Co., 1907, Philadelphia and New York.

[4] *Trans. Nat. Ass. Study and Prevention Tuberculosis*, 1905, 1, p. 166.

of 130 cases of pulmonary tuberculosis of which this paper is a report, I am convinced that blood cultures give definite, positive proof that secondary invading organisms are present in the blood stream of a large percentage of individuals afflicted with pulmonary tuberculosis, and that the organisms so present play an extensive rôle in the production of the symptoms. Before proceeding to give details of the methods and results of my own work however, it may be well to analyze the previous attempts in this direction.

By way of introduction it may be stated that the investigation by means of blood cultures fall into two groups, namely, those of the earlier workers and those of relatively recent date. The early investigators obtained a high percentage of positive results. They secured the blood by pricking the ear or finger, and allowing a few drops to fall through the air into culture media. The chance for air and skin contamination was great, and the number of staphylococci (chiefly albus) isolated, indicates that the large percentage of their positive findings was due to such contaminations. On the other hand, the later investigators, using a more trustworthy technic, drawing the blood directly from a vein under attempted aseptic conditions, have shown a very low percentage of positive results—about 2.5 per cent.

Jakowski,[1] puncturing the finger and allowing the blood to drop into culture media, secured seven positive results in nine observations. Two of the positive cultures gave streptococcus; one, streptococcus and staphylococcus together; and four, staphylococcus.

Hewelke[2] found organisms in 14 out of 21 cases. Ten were staphylococcus; one case showed a diplococcus and one case a non-pathogenic organism, either a coccus or a short bacillus. In another series, using venous puncture, he obtained three positive results out of 13. These were all non-liquefying white cocci.

Petruschky[3] drew a definite amount of blood, and injected mice and inoculated culture media. He found streptococci in one case out of eight.

Sittman,[4] drawing one c.c. of blood from the vein at the elbow in four cases, obtained *Staphylococcus aureus* in three cases and *Staphylococcus albus* in one.

Schabad,[5] using the same method as Sittman, recovered *Staphylococcus albus* in one case out of three.

Kraus,[6] in 14 observations, obtained the *Staphylococcus albus* in one case.

Hirschlaff[7] found a staphylococcus in four of 35 cases.

Von Michaelis and Meyer[8] found bacteria in blood cultures from eight cases in

[1] *Centralbl. f. Bakt.*, 1893, 14, p. 762.
[2] *Ibid.*, 1896, 19, p. 563.
[3] *Deut. med. Wchnschr.*, 1895, 19, p. 317.
[4] *Deut. Arch. f. klin. Med.*, 1894, 53, p. 323.
[5] *Ztschr. f. klin. Med.*, 1897, 33, p. 476.
[6] *Ztschr. f. Heilkunde*, 17, p. 117.
[7] *Deut. med. Wchnschr.*, 1897, 13, p. 766.
[8] *Charitéannalen*, 1897, 22, p. 150.

10. Five of the organisms were staphylococcus, one was a diplobacillus, one a gram-positive diplococcus, and one a streptococcus. The blood was taken shortly before death—four, three, two, nine, and 14 days, for those recorded.

A. Fraenkel,[1] using Sittman's method, obtained negative results in all of 20 cases.

Schroeder and Naegelsbach,[2] putting one c.c. of blood into broth, obtained negative results in all of eight cases. The patients were far advanced and all died within one month after the taking of the blood.

Straus[3] found the blood sterile in 19 cases, although they were all in the last stages; most of them with marked remittent fevers.

Lasker,[4] plating two c.c. of blood in agar, obtained but one positive result in 68 cases. This case showed many streptococci.

Lemierre[5] found the blood cultures all negative from eight cases in the last stages.

Teissier,[6] investigating 53 cases, obtained nine positive results. He drew one c.c. of blood from an arm vein, and divided it among several tubes of gelatin and broth. Of the nine positive results, two were *Staphylococcus aureus*, three, streptococcus, and four were *Staphylococcus albus*.

Jockmann,[7] examining 40 cases, drew 20 c.c. of blood from the vein at the elbow, distributed this among six or seven tubes of agar at 45° C.; after shaking thoroughly, the contents were poured into Petri dishes and incubated at 37° C. All the results were negative. The patients showed various temperatures. Some were of a remittent type and cavities were present in almost all cases.

Panichi[8] found pneumococci in four cases of some 35 examined. He drew scarcely one c.c. of blood. These were all advanced cases; but one lived for seven months after the blood was taken. Panichi concludes that bacteremia may occur before the agonal period and be intercurrent. Pneumococci were found in one case that did not give a history of previous pneumonia, and he concludes that it was an organism coming from a cavity.

Benohr,[9] investigating 187 cases of tuberculosis, making 241 examinations, obtained four positive results. He drew 20 c.c. of blood and plated it in glycerin agar.

F. Reiche,[10] making 365 examinations on 288 cases of high fever in terminal stages, took 15 to 20 c.c. of blood and obtained 1.65 per cent positive results.

The workers following Sittman, for the most part, drew blood directly from a vein and their results are therefore to be held the more reliable in that the possibilities of contamination were greatly reduced. However, many of the positive results of these workers undoubtedly included contaminations, namely staphylococci from the skin. If, however, the staphylococcus findings are eliminated from the results of Tessier, von Michaelis and Meyer, Hirschlaff,

[1] *Berl. klin. Wchnschr.*, 1898, 35, p. 345.
[3] *Semaine méd.*, 1894, 14, p. 253.
[2] *Münch. med. Wchnschr.*, 1899, 46, p. 1339.
[4] *Deut. Aertzte-Zeitung*, 1901, 1, p. 27.
[5] *Bull. et mém. Soc. Med. de l'Hôp. de Paris*, 1903, 20, p. 1437.
[6] *Jour. de physiol. gén.*, 1901, 3, p. 223.
[7] *Deut. Arch. f. klin. Med.*, 1905, 83, p. 558.
[8] *Berl. klin. Wchnschr.*, 1908, 41, p. 1840.
[9] *Mitt. a. d. hamb. Staats-Krankenanst.*, 1908, 13, p. 323.
[10] *Med. Klinik*, 1909, 5, p. 1962.

Kraus, Lasker, Schabad, Sittman, Panichi, Jockmann, Reiche, and Benohr and others, I believe their positive results are reliable; for, as discussed later, streptococci and pneumococci are not organisms furnished by skin contamination. Excluding the staphylococci, however, the results of these later workers show a very low percentage of positive cultures and it has been concluded that if secondary infection is of importance at all, its influence is due to soluble toxins escaping from a localized infection in the lung, and not to a bacteremia.

My own experiments, as stated, lead me to quite the opposite conclusion, namely, that the invasion of the blood stream by pyogenic organisms is frequent in pulmonary tuberculosis.

In carrying on my work I have given emphasis to three main points: (1) the drawing of a large quantity of blood under the most favorable aseptic conditions, (2) the systematic inoculation of the most favorable media with considerable amounts of this blood, and (3) the rigid exclusion as positive cultural results of all organisms having a possible origin in skin or air contamination.

To cover the first point the following technic was employed in obtaining the blood for cultural purposes:

From 5 to 20 c.c. of blood, usually the latter amount, was drawn from an arm vein by means of a glass aspirating bulb of about 25 c.c. capacity. A cotton plug was placed within one of the ends and a piece of heavy rubber tubing about six inches long attached to this end. A second, shorter piece of rubber tubing was attached to the other end. An antitoxin needle (No. 18 caliber) was inserted into the distal end of the shorter piece of tubing and the tubing tightened about the hilt of the needle by binding with a rubber band. The needle was protected by slipping a test tube over the end.

Shortly before the bleeding, the whole aspirator, including the tubings and needle with a test tube over it, was wrapped in a towel and sterilized in the autoclave at 120° C. for at least 15 minutes.

In drawing the blood the upper arm was encircled tightly with a rubber bandage, the cubital region was scrubbed vigorously with alcohol,[1] anesthetized quickly with ethyl chloride, and the venous puncture made. When the blood commenced to flow into the aspirator, the compression of the upper arm was relieved, and the blood aspirated into the bulb by suction through the heavy rubber tube attached to the cotton-plugged end of the aspirator.

[1] Various methods of skin sterilization were tried, but none was found that was more efficient than the scrubbing with alcohol. In a number of cases the skin of the cubital region was scrubbed thoroughly with green soap, and rinsed with five per cent carbolic acid and 1/1000 mercury bichloride. In another group of cases the cubital region of the patient's arm was scrubbed with green soap and painted with tincture of iodin.

The percentage of cultures showing *Staphylococcus albus* after these methods of skin preparation was as great as in the cases in which simple cleansing of the skin with alcohol was used.

As soon as the required amount of blood had been withdrawn, the needle was removed from the vein, a wire sterilized in the flame was inserted to occlude the lumen of the needle, and the whole needle then heated to redness. This, with the resulting coagulation of the blood, effected a sealing of the needle.

In making the transfer from the aspirator to the culture media, the short rubber connection and the needle were detached and the end of the aspirator heated thoroughly in the flame. The corks of the culture tubes and flasks were flamed, the corks removed by an assistant, the necks of the tubes and flasks heated thoroughly in the· the flame and the desired amount of blood was then transferred quickly from the aspirator to culture flask. The end of the aspirator was flamed again before more blood was transferred to a second culture flask. In spite of a rigorous enforcement of this technic as a precaution against contamination in drawing the blood and in transferring it to the culture media, contamination by staphylococci occurred in 10 per cent of the cases. The staphylococcus undoubtedly represented a skin contamination and in a control series of 21 blood cultures made on normal individuals, a similar contamination occurred in two instances.

In the attempt to grow organisms from the blood obtained in the manner above given, the following cultural methods were employed. Five c.c. of agar was sterilized in eight-ounce flat flasks, and immediately before using, the agar was melted and cooled to 40° C. From one to two c.c. of blood was introduced into each of one, two or three of these flasks containing melted agar, and after the blood and agar were thoroughly mixed by gently agitating, the flasks were laid on the side and the agar allowed to harden, giving a large layer of blood agar, about $\frac{1}{8}$ in. in depth. The flasks were next put in the incubator at 37° C.

In a majority of the cases cultures were also made in broth,[1] usually five c.c. of blood being put into 50 c.c. of broth. No particular attention was paid to an exact dilution other than to avoid a concentration greater than one to 10. As a usual routine two or three such inoculations of broth were made. In a few cases flasks of litmus milk were used, but the broth was found to be much more satisfactory.

The residuum of the blood not employed in the above inoculations was incubated in the sealed aspirator or in a sterile test tube.

After 24 hours at 37° C., the blood-agar flasks were examined with a low-power lens with especial reference to the presence or absence of deep colonies showing hemolytic zones. In the case of a positive finding, typical colonies were transferred to blood-agar slants. The broth was examined after 48 hours. Microscopic examination was made of smears stained with simple gentian violet and by Gram's method. Blood-agar plates were inoculated with one c.c. of the 48-hour broth regardless of whether the microscopic findings were positive or negative. In a considerable number of instances, milk tubes also were inoculated from the broth. The full blood, incubated for 24 hours in the aspirator, was examined microscopically for organisms, and whether the finding was positive or negative, transfers were made at once to blood-agar slants. After incubation of the residuum of full blood for an additional 24 hours (total 48) this was plated in blood agar.

The blood-agar transfers obtained as above indicated from the original blood agar and broth and from the incubated full blood, were, in turn, incubated at 37° C. for 24 hours. The resulting growths were, in all instances, streptococci, pneumococci, or staphylococci.

[1] Mallory and Wright, *Technic.*, 3d ed., Saunders & Co., 1904, pp. 71, 73, 74.

1. Cultures showing a clear, dewlike growth, confined to the needle track; or scattered, clear, fine, pin-point colonies were examined microscopically for a characteristic arrangement of the organisms in chains or in pairs. Transfers of individual colonies were made to plain-agar, potato, gelatin, milk, broth, dextrose-agar, and serum-inulin-agar, or serum-inulin-water and blood-agar and the cultural study of the fully isolated organism was continued in each instance until its identity was established. Daily descriptions of the growths were recorded for six consecutive days.

2. The 24-hour blood-agar cultures, which in contrast to those described above, presented an abundant surface growth, were examined microscopically for staphylococci, and were transferred at once to the usual series of culture media. An organism forming a white or yellow growth on plain agar, distinct growth on potato, liquefaction of gelatin, a considerable cloudiness in broth, or a white or yellow surface-growth on dextrose agar, was in all instances assumed to be a staphylococcus, was identified as such and was discarded as presumably representing a skin contamination.

As above stated the only organisms obtained in the original cultures of the entire series of examinations were staphylococci, streptococci, and pneumococci; and inasmuch as the staphylococci were eliminated, the results recorded as representing actually positive blood cultures have to do only with streptococci and pneumococci. On this basis alone, however, positive results were obtained in 46 per cent of the cases examined (60 in 130). The streptococcus was found in 36 cases and the pneumococcus in 24 cases.

It is not possible within the scope of this paper to give in detail the bacteriological results for the entire number of positive cases. The following six instances, however, serve as examples of the application of the methods outlined above and the results obtained.

"P."—Incipient pulmonary tuberculosis. Temperature normal. Ambulatory. Disease passive. Hemorrhagic history. Family history indicates marked predisposition to tuberculosis. Marked infiltration of right lung; moderate impairment of upper portion of left lung.

Cubital region of arm scrubbed with green soap and painted with iodin. Immediately before making venous puncture, operator's hands, covered with gloves, were dipped in 95 per cent carbolic acid and rinsed in mercury bichloride, 1/1000. Ten c.c. of blood was drawn from median basilic vein and two c.c. transferred to each of four flasks containing five c.c. of melted agar cooled to 40° C. Agar and blood were thoroughly mixed, and plates made by laying the flasks on the side. When agar had hardened the flasks were put in the incubator at 37° C. After 18 hours, numerous greenish colonies with hemolytic zones were seen on the plates.[1] Several such colonies were transferred to blood-agar slants. Blood-agar slants after 24 hours showed discrete, greenish, hemolytic colonies, shown by microscopic examination to be composed

[1] After 72 hours, plates were somewhat overrun with surface growth of white colonies. Smear of this latter growth showed large cocci in bunches. Identified as *Staphylococcus albus*.

of gram-positive cocci arranged in long chains. Several of these discrete colonies were transferred from the agar slants to a series of media with the following results:

Subcultures after 24 Hours.

Blood agar: Few very small, dewlike, clear colonies. No increase in growth after 96 hours.

Plain agar: Same as blood agar, only much less extensive, practically invisible. No increase in three days, slightly increased in four days.

Potato: No growth in six days.

Gelatin: No growth in six days (room temperature).

Litmus milk: No apparent change.

Broth: No apparent growth.

Dextrose agar: No surface growth; questionable growth along stab.

Serum-inulin-agar: No change.

Diagnosis: Streptococcus.[1]

"D."—Advanced pulmonary tuberculosis. Condition passive. Ambulatory. Hemorrhagic history. Moderate infiltration of both lungs.

Cubital region of arm scrubbed with alcohol and 20 c.c. of blood drawn from median basilic vein. Transferred two c.c. immediately to an agar flask containing five c.c. melted agar cooled to 40° C. and transferred five c.c. to each of three flasks containing 50 c.c. of broth. The remaining blood was sealed in the aspirator and incubated without diluting.

Subcultures after 24 Hours.

Blood agar: Showed many greenish colonies with hemolytic zones. Several colonies were transferred to blood-agar slants. Smears from 18-hour blood-agar slants showed diplococci in short chains and cocci in bunches. Growth on various media showed streptococci with *Staphylococcus-albus* contamination. A colony from the original blood-agar decolorized milk in 24 hours, and the smear showed many capsulated diplococci; some in short chains. Pure culture.

Broth: After 72 hours transfers were made to blood-agar slants. These subcultures, after 24 hours, showed fine, compact, opaque growths. Smear showed diplococci in short chains and groups. The cultural characteristics, staining reactions, and morphological arrangement of the organism isolated from flask No. 2 identified it as a streptococcus.

Full blood: Microscopic examination after 48 hours of the three c.c. of undiluted blood incubated in the sealed aspirator showed diplococci in short chains. Transfers were made to blood-agar slants, which after 24 hours showed slight, compact, greyish-looking growth. Smears from the same showed gram-positive diplococci in long chains,

[1] The differentiation of the streptococci from the pneumococci—in so far as that is possible—was based upon the following criteria: (1) Appearance of colonies on blood-agar slants; (2) Appearance of colonies on blood-agar plates, green colonies with imperfect hemolysis (fuzzy border) were considered pneumococci, while clear or opaque colonies showing zones of sharply demarcated hemolysis were considered streptococci if showing other suggestive characteristics; (3) Chain formation on blood agar and in broth; (4) Reaction on serum-inulin-agar (Ruediger) or in serum-inulin-water (Hiss). Growth in gelatin capsule formation, growth on plain agar, and morphology, were taken into account in making a differentiation, although they were not considered of distinct diagnostic value. It need hardly be added that applying all possible criteria, the validity of an absolute decision, as to which one of these two groups a given organism belonged to, was questionable.

which were plated in blood agar, with resulting colonies showing distinct clear zones of hemolysis. Transfers were made to a series of media with the following results:

Blood agar: 24 hours—fine, dewlike, compact growth along needle track; 48 hours—growth slightly increased.

Potato: No growth.

Litmus milk: 24 hours—slightly acid(?); 72 hours—acidified and coagulated.

Gelatin: No growth in three days.

Broth: No growth. Smears after three days negative.

Dextrose agar: No surface growth. Growth along stab(?).

Serum-inulin-water: No acid production.

Diagnosis: Streptococcus.

"C."—Advanced pulmonary tuberculosis. Disease active. Ambulatory. Marked infiltration of right lung.

Twenty c.c. blood was drawn and two c.c. was transferred to each of two flasks containing five c.c melted agar cooled to 40° C., five c.c. were put in each of two flasks containing 50 c.c. of plain broth, and remainder of the blood was put in a sterile test tube.

Blood agar: Both were sterile after 48 hours.

Broth: Microscopic examination of flask No. 1 showed gram-positive diplococci in short chains. Transfers were made to other culture media after 48 hours with results as follows:

Blood agar: Very scant, scarcely visible growth of fine, dewlike colonies after 24 hours; slightly increased after 72 hours. Smear showed gram-positive diplococci. Pure culture.

Plain agar: No growth.

Potato: No growth.

Gelatin: No growth.

Milk: 24 hours—acid; smears showed gram-positive diplococci. Pure culture.

Broth: Clear, but with pellicle. Smear showed moderately long chains of gram-positive cocci (12–24 cocci in a chain). Bacillus seen. Second tube was inoculated; slightly cloudy in three days. Gram-positive diplococci in chains were seen. Pure culture.

Dextrose agar: No growth.

Serum-inulin-water (Hiss): Acid in four days.

Diagnosis: Pneumococcus.

"G."—Far-advanced pulmonary tuberculosis. Condition very active. Large cavity. Several bad hemorrhages. Bed-ridden. Marked infiltration of both lungs, more pronounced in left.

25 c.c. of blood was drawn with usual technic and three c.c. of blood was transferred at the bedside to each of four flasks containing five c.c. of melted agar cooled to 40° C. Five c.c. of blood was transferred to each of two flasks of 50 c.c. broth. The remaining blood was sealed in aspirator and incubated undiluted.

Blood agar: After 24 hours the flasks showed many small, greenish colonies imbedded in the blood agar and also a surface contamination. Transfers were made from several of the deep colonies to blood-agar slants but the subcultures showed contamination with staphylococci. Discarded for pure cultures obtained from the full blood as below.

Broth: After four days the broth showed typical lanceolate diplococci, gram positive. No subcultures were made.

Full blood: Smears showed diplococci in long chains at the end of 24 hours. Subcultures were made on a series of media with the following results:

Blood agar: Fine, clear, dewlike, moist, elevated colonies; growth confined to needle track. Growth increased after 48 hours. Smear showed gram-positive diplococci in long chains.

Plain agar: 24 hours—very scant, almost invisible growth; 48 hours—growth somewhat increased; six days—no further change. Smear, after 24 hours, showed long chains of diplococci.

Potato: No growth.

Gelatin: No growth.

Litmus milk: 48 hours—acid. Not coagulated in six days.

Broth: 48 hours—visible growth. Slight cloudiness with granular sediment in six days.

Dextrose agar: Scant surface growth(?); scant growth along stab. No gas.

Serum-inulin-agar: Acid production in three days.

Diagnosis: Either pneumococcus or streptococcus—doubtful as to which.

"K."—Far-advanced pulmonary tuberculosis. Very active. Bed-ridden. Marked infiltration of right lung. Cavity.

Five c.c. blood was drawn and about two c.c. of blood run into a flask containing five c.c. of melted agar cooled to 40° C. After the blood was mixed with agar it was plated in a thin layer by laying flask on its side. The remainder of the full blood was sealed in the aspirator and incubated.

Blood agar: Negative after 48 hours.

Full blood: A transfer was made from the full blood to a blood-agar slant, after 24 hours. Twenty-four hour blood-agar slant showed scattered, small, greyish, opaque colonies. Smear showed cocci in chains. Transferred to other media.

Subcultures.

Blood agar: Scattered, small, greyish, opaque, elevated, moist colonies after 24 hours. Very scant growth. Not increased in five days.

Plain agar: Very scant growth of clear, dewlike colonies. Growth slightly increased in 72 hours.

Potato: No growth in six days.

Gelatin: No growth in six days.

Litmus milk: Acid in 24 hours, fine coagulum in four days.

Broth: Fine, granular sediment; fluid clear. Smear after three days showed gram-positive cocci in long chains.

Dextrose agar: Scant, clear, surface growth. Slight growth along stab(?).

Litmus-inulin-agar: No acid.

Diagnosis: Streptococcus.

Blood culture made again one week later and the streptococci were again recovered.

"V."—Moderately advanced pulmonary tuberculosis. Active. Ambulatory. No hemorrhage. Marked infiltration of right lung. No cavities.

About 10 c.c. of blood was drawn and three c.c. was transferred immediately to each of three flasks containing five c.c. of melted agar cooled to 40° C. After mixing blood thoroughly with agar, plates were made by laying flasks on the side. After 24 hours at 37° C., several green colonies surrounded by clear zones appeared in two of the flasks, the third flask remaining sterile. Some of these colonies were transferred

to blood-agar slants, and from these, after 24 hours, subcultures were made on various media with results as follows:

Blood agar: 24 hours. Scattered, flat, green colonies, which clung tenaciously to the culture medium.[1] Colonies had a truncated-cone appearance. A smear showed capsulated diplococci. Growth increased after 48 hours. No further change in six days.

Plain agar: Very small, clear, scattered colonies in 24 hours. No change in six days.

Potato: No growth.

Gelatin: No growth.

Litmus milk: Acid production at 24 hours. Coagulation in three days. Smear showed gram-positive diplococci, lanceolate in shape.

Broth: No growth.

Dextrose agar: Good growth along needle track. No surface growth.

Diagnosis: Pneumococcus.

The details of the above six cases are representative of the findings in all of the 60 cases from which pneumococci or streptococci were isolated.

Twenty-three of the 60 strains isolated were tested as to their pathogenicity for mice, and 16 were found lethal.

Fifteen strains of the 60 isolated were employed also in conjunction with the homologous sera to determine whether or not the presence of the organism in the blood stream had modified the opsonizing value of the serum. In the case of 10 of the 15 sera (66 per cent) the opsonic index for the homologous organism was abnormal, namely, below 0.8 or above 1.2.

Number	Organism	Opsonic Index
1	Streptococcus	0.85
2	Pneumococcus	1.3
3	Pneumococcus	0.7
4	Streptococcus	0.95
5	Pneumococcus	0.5
6	Pneumococcus	0.7
7	Streptococcus	0.8
8	Pneumococcus	0.75
9	Pneumococcus	0.5
10	Pneumococcus	1.3
11	Streptococcus	0.8
12	Streptococcus	0.75
13	Streptococcus	0.7
14	Streptococcus	0.8
15	Streptococcus	0.7

As mentioned previously, a total of 130 cases, including instances of all stages of pulmonary tuberculosis, from passive incipient involvement to far-advanced active processes with cavity forma-

[1] In several cultures, organisms were isolated which displayed this characteristic.

tion, were examined. Streptococci and pneumococci were recovered from the blood in 60. The relation of the bacteriological findings to the various stages of the disease are given in the following summary:

Classification	Number of Cases Examined	Number of Positive Blood Cultures	Percentage of Positive Blood Cultures
Incipient..........	12	2	16
Advanced..........	99	45	45
Far advanced......	19	13	68
Total.........	130	60	46

An analysis of this summary shows that positive blood cultures were obtained in a relatively small number of cases classed as "incipient," whereas the percentage was almost three times as great among the "advanced" cases. From the "far-advanced" cases the percentage of positive results was almost twice that among the "advanced" cases, and four times that of the "incipient" cases; 58 of the 60 positive cultures were obtained from "advanced" and "far-advanced" cases.

The general relation of the bacteriological findings to the grade of fever displayed by the host is expressed in the following statement:

Grade of Fever	Number of Cases Examined	Number of Positive Blood Cultures	Percentage of Positive Blood Cultures
Afternoon temperature below 100° F....	65	22	34
Afternoon temperature above 100° F....	65	38	58

From this it is seen that approximately two-thirds of the positive cultures were obtained from individuals showing a very distinct afternoon fever.

Could it be shown that the streptococci and pneumococci obtained in the above results have a possible source in air or skin contamination, as is actually the case with the staphylococci for instance, the above findings would be of little or no import in the discussion of secondary invaders. Hektoen[1] has rightly pointed out, however, that such contaminations with organisms other than the staphylococci, if occurring at all, are of such extremely rare occurrence in cultures with blood obtained by venous puncture as

[1] *Jour. Am. M. Ass.*, 1903, 40, p. 683.

to be a negligible factor. Rarely, indeed, is it possible to obtain streptococci by direct inoculations from the skin, even in the absence of surface sterilization. Thus Weaver,[1] attempting to cultivate this organism from the skin in a group of 18 scarlet-fever cases, was successful in but one case, and Dreyer, in a similar group of 30 cases, failed to cultivate the streptococcus in a single instance.

To control this point under fully parallel conditions, however, blood cultures were made from 21 normal individuals, employing exactly the same technic as that used in the case of the 130 tuberculous patients, even to the amount of blood withdrawn—10 to 20 c.c. The results obtained were in accord with the above statements, namely: Neither streptococci nor pneumococci were encountered in a single instance; whereas *Staphylococcus pyogenes albus* was present in two cases.[2]

In view of these facts, the conclusion seems warranted that the pneumococci and streptococci isolated by the blood-culture method used in the present instance had their origin not in an extraneous source, but actually in the circulating blood stream.

The high percentage of positive results in the series of cases here reported contrasts sharply, to be sure, with the results of previous workers who have employed the blood-culture method in studying secondary infections in pulmonary tuberculosis. The explanation of this difference I do not seek to establish other than to point out that in the present series a large number of cases was examined, a large amount of blood was used, and finally the cultural conditions most favorable to the growth of the organisms in question were rigorously maintained.

The isolation of the pneumococcus or the streptococcus from the blood stream of more than one-third of the cases examined leads me to the conclusion that not only are true secondary invading organisms of frequent occurrence in pulmonary tuberculosis, but further, that in many instances these organisms, entering the blood stream, constitute a complication of extensive pathological significance.

[1] Cited by Hektoen.

[2] The skin of the forearm of six normal individuals was entered with sterile needles which were then plated in blood agar. In two instances, there was a growth of the *Staphylococcus pyogenes albus*, the other four plates remaining sterile.

INFECTION BY THE GAS BACILLUS IN COAL-MINES.*

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The discovery by Professor Welch in 1891 of *B. welchii*, and the observed relationship of this organism to the production of gas in the tissues was the first definite evidence that the inflation of tissues before and after death was commonly due to bacterial invasion. The presence of free gas in various parts of the body has been commented upon in the literature by many of the older writers, and for some time it strengthened the belief that air was an important constituent of the circulation. Simple putrefaction was suggested by others as the cause of the accumulation of this gas. On the other hand, prior to Welch's discovery, we have the observations of Pasteur, Koch, and their pupils, which indicate that a type of organism was found and known to them which in all probability belonged to the *B. welchii* group.

Welch and Nuttall published their first report in 1892. Then there followed a series of observations from the laboratories at Johns Hopkins Hospital indicating the wide distribution of this organism in nature and disease. Almost simultaneously there appeared in Germany and France similar observations by Fraenkel, Ernst, Goebel, Dobbin, and others. The importance of this type of infection was emphasized by each, and the danger of its presence in deep wounds, particularly when associated with other pyogenic bacteria, was appreciated by the surgeon. With, however, the improved technic in bacteriology, along with keen interest for the isolation of new anaerobic forms, we find that several investigators determined the presence of organisms, simulating in many respects *B. welchii*, but on account of finding them in new locations and of slight, possibly transient variations, applied a new nomenclature. Thus Fraenkel, finding a large anaerobic bacillus in gaseous phlegmons gave it the name *B. phlegmones emphysematosae;* Veillon and Zuber isolated bacilli from cases of otitis media, pulmonary gangrene, mastoiditis, appendicitis, and pelvic abscess which they

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called *B. perfringens;* Lindenthal, in 1897, isolated an organism from the emphysematous tissues about the vagina, to which he gave the name *B. emphysematis vaginae.* Likewise to an organism isolated from milk and water and having characters of *B. welchii,* Schattenfroh and Grassberger gave the name *Granulobacillus saccharobutyricus immobilis liquefaciens.*

No general systematic study has been undertaken to determine which of these organisms are identical with or belong to the group of organisms of which *B. welchii* is the important representative. It is probable that the slight variations which have been observed in these somewhat similar organisms are real, but it is plain that, with the meager information obtainable for many of them, no very definite statement can be made for their isolated identity. To this uncertain class might be added *B. vaginae* (Kruse), *B. cadaveris* (Sternberg), and *B. cadaveris butyricus* (Buday). It is also evident that many of the cases of human infection which have been described in America as malignant edema are instances of infection by the *B. welchii.* Clinically, some cases presenting features of malignant edema with progressive gangrene, in which very little gas can be demonstrated in the tissues, show on bacteriological examination the presence of the "gas bacillus" along with other organisms.

Some confusion has also arisen between *B. welchii* and *B. enteriditis sporogenes* (Klein). The latter organism has been isolated by Klein from a great number of sources (sewage, manure, feces, street dust, milk, etc.). This organism has certain characters which distinguish it from the organism of Welch, particularly to be observed in the presence of flagella and motility. Klein reports the ease with which spores are produced in tissues; but this appears to vary with certain strains, and in cultures is equally difficult to obtain as with *B. welchii.* Again it is pointed out that *B. enteriditis sporogenes* is highly virulent, but this too is not constant, as the cultures of the organism may rapidly lose their virulence. There is, however, a striking resemblance between the organisms of Klein and that of Welch in the production of gas in the animal tissues. By using the same procedure as Welch of inoculating the organism into the veins and then killing the animal

and incubating, Klein has found that *B. enteriditis sporogenes* produces much gaseous emphysema throughout the body. Another feature in which these organisms resemble each other is the sparse growth of thin translucent colonies on surface agar.

A most comprehensive study of the "Morbid Conditions Caused by *Bacillus aerogenes capsulatus*" is given by Professor Welch in his Shattuck Lecture of 1900. It is pointed out by Welch that the duplication of nomenclature has led to much confusion in the discussion of the bacteriology of emphysematous gangrene. This we have met with in our study, but it has also appeared to us that *B. welchii* constitutes a group of organisms, each of which may produce gas in tissues, and have some slight variation in morphological or cultural features.

Our attention was attracted to this infection by the unusual frequency of emphysematous gangrene occurring in the surgical wards of the hospitals in Pittsburgh. For the clinical data we are indebted to Drs. R. W. Stewart, J. J. Buchanan, G. L. Hays, A. Stewart, and J. P. Griffith, surgeons at the Mercy Hospital.

The reports of the cases comprising this study have been taken from the records of the Mercy Hospital at Pittsburgh. There are 36 cases in all, in which both the clinical and bacteriological examinations demonstrated the presence of *B. welchii* or one of the members of this group. A number of other cases in which a satisfactory analysis of the bacterial infection was not forthcoming were not included, although we felt that some of them at least were infections by the gas bacillus. These latter cases gave clinical evidence of gas in the tissues at the point of injury, some also showing indurative edema with gangrene, but a bacteriological examination had been omitted. These cases must remain in the undetermined class, but we feel with Welch that the production of gaseous gangrene in life is in the majority of cases due to the infection of *B. welchii* or one of the members of this group, and that gas formation in tissues by *B. coli* remains unproved, save possibly in a few reported cases of diabetes. Concerning infections by the bacillus of malignant edema in man, we have had no experience, and believe that authentic cases of this condition in America are extremely rare.

As the hospital from which our records are taken does not admit

maternity cases we have no instance in this series of uterine infections by *B. welchii*.

TABLE 1.

Number	Age	Sex	Occupation	Nature of Accident	Nature of Wound	Result
1	27	Male	Bridge-builder	Fall from bridge	Comp'd fracture of arm	Recovery
2	31	Male	Telephone lineman	Fall from pole	Comp'd fracture of arm	Recovery
3	20	Male	Coal-miner	Run over by car in mine	Comp'd fracture of ankle	Recovery
4	14	Male	Coal-miner	Run over by car in mine	Comp'd fracture of both thighs	Recovery
5	28	Male	Coal-miner	Fall of slate in mine	Comp'd fracture of fibula and tibia	Death
6	24	Male	Laborer	Accident at steel works	Comp'd fracture of leg	Recovery
7	42	Male	Coal-miner	Fall of slate in mine	Crush of foot (open wound)	Death
8	16	Male	Mill worker	Accident at steel works	Crush of foot (open wound)	Recovery
9	72	Male	Coal-miner	Crush by cars in mine	Laceration of leg (open)	Death
10	31	Male	Coal-miner	Crush in mine	Comp'd fracture of leg	Death
11	19	Male	Brakeman	Crush by train	Comp'd fracture of hand	Death
12	54	Male	Coal-miner	Crush in mine	Comp'd fracture of leg	Death
13	29	Male	Coal-miner	Crush by cars in mine	Comp'd fracture of leg	Recovery
14	25	Male	Coal-miner	Crush by car in mine	Comp'd fracture of femur	Death
15	25	Male	Electrician	Fall from scaffold at a mill	Comp'd fracture of radius	Recovery
16	23	Male	Coal-miner	Fall of slate in mine	Comp'd fracture of fibula	Recovery
17	45	Male	Coal-miner	Fall of slate in mine	Laceration of foot	Recovery
18	31	Male	Coal-miner	Crush by car in mine	Left thigh almost severed	Recovery
19	21	Female	Housewife	Fall from porch	Cut forearm in glass on ground	Recovery
20	19	Male	Laborer	Explosion of powder	Fracture of leg with laceration	Recovery
21	23	Male	Mine driver	Crush by car in mine	Comp'd fracture of both legs	Recovery
22	36	Male	Mill worker	Crush in mill	Comp'd fracture of both legs	Recovery
23	17	Male	Coal-miner	Crush by car in mine	Comp'd fracture of leg	Death
24	28	Male	Laborer	Fall into ditch 15 ft.	Comp'd fracture of femur	Death
25	9	Male	School boy	Fall from ladder	Comp'd fracture of forearm	Recovery
26	23	Male	Machinist	Fall from box-car	Comp'd fracture of humerus	Death
27	39	Male	Coal-miner	Injured by mine truck	Comp'd fracture of tibia	Recovery
28	32	Male	Coal-miner	Crush in mine	Thigh lacerated	Death
29	9	Male	School boy	Fall from tree	Comp'd fracture of forearm	Death
30	40	Male	Laborer	Train accident	Crush of left arm	Recovery
31	38	Male	Coal-miner	Fall of slate in mine	Comp'd fracture of ankle	Recovery
32	30	Male	Laborer	Run over by street car	Crush of left leg	Death
33	15	Male	School boy	Run over by train	Crush of legs	Recovery
34	61	Male	Coal-miner	Crush by car in mine	Laceration of thigh	Death
35	24	Male	Coal-miner	Crush by car in mine	Crush of heel	Recovery
36	24	Male	Coal-miner	Fall under car in mine	Comp'd fracture of both legs	Death

Gas present in tissues in all cases.

In summarizing the above 36 cases we find that there were 35 males and one female. The youngest individual was nine years and the oldest 72 years. The average age was 31 years. Every case in the series had a severe external wound. There were 23 cases with compound fractures. Of these, 16 were of the lower and seven of the upper extremity. The remaining 13 cases consisted of severe lacerated flesh wounds in which no bones were broken but in which extensive areas were laid bare to infection of all kinds. The very nature of these accidents where great mechanical injury was received, led, in nearly all cases, to the fall of the individual to the ground. It is this feature, the pollution of the wound by the infected soil, in many instances the dirt being ground into the tissues, which makes these lesions very susceptible to this form of infection.

More interesting, however, is the evidence of occupation susceptibility. Of the 36 cases which we here report, there were 20 coal-miners who received their wounds while at work in the mines. The accidents leading to the extensive lacerations were of two kinds: (1) the falling of the slate from the roof of the mine upon the workman; or (2) crush-accidents between or under the mine cars. In either case the individual received lacerations with large open wounds which readily became infected by the soil of the mine. Temporary first-aid treatment was received in the mine. The severe wounds were later given hospital treatment. Gas in the tissues about the wound was observed to occur as early as 12 hours, and as late as four days, after the accident. Of the entire 36 cases reported, 40 per cent proved fatal, while of those receiving their injury in the coal mines, the mortality was 50 per cent.

Of the remaining 16 cases not working in the mines there were:

Laborers	5	Electrician	1
School boys	3	Brakeman	1
Mill workers	3	Telephone lineman	1
Bridge-builder	1	Housewife	1

In all instances but one, the injuries were received while the individual was out-of-doors, and in the majority of these 16 cases the wounds were obtained on the street or some driveway or in a garden. Two of the school boys were injured in a fall in a garden,

from a ladder and a tree respectively. The injury, with gas-bacillus infection in a woman, was received in a fall from a porch on to the garden soil. Three individuals were injured in railroad accidents, in which the lacerated wounds were soiled by the earth along the railroad tracks. One case developed tetanus after the appearance of gaseous emphysema.

It is significant in all of these cases that the wounds were infected by earth, dirt, or materials which themselves were open to fecal pollution from either man or animals. It is obvious that the highways are repeatedly reinfected by various pathogenic organisms, and although sunlight and rain greatly assist in removing from the surface the presence of living bacteria, a great variation exists in the extent of this disinfection. The presence of moisture, shade, and porous organic substances assists greatly in preventing the destruction of many bacteria. Great variation, too, exists among the individual organisms in their resistance to external destructive influences, so that soil and dust, although subject to climatic changes, commonly contain an abundance of spore-bearing bacteria and such other organisms which are not so readily affected by variable temperature and light.

It has been demonstrated by many observers that *B. welchii* and its closely related forms are almost constant inhabitants of the intestines of both man and animals. Welch and Flexner, Clopton and Howard have isolated this bacillus from the intestines of man, while Welch has also demonstrated its presence in the bowel of rabbits, dogs, and swine, and Houston obtained it in cow dung. Walker isolated the organism from the dust of hospital wards; Harris has found it in the contents of cesspools, and Fraenkel has obtained it in a splinter of wood which had led to an emphysematous infection of a wound. We have isolated a gas bacillus with characters of *B. welchii* from rich garden earth, and we have also obtained it from the tissues of a cadaver undergoing putrefaction in whom no external wound of entry existed. It is evident, therefore, that this bacillus is widely disseminated in nature and that the contamination of the soil will continue to exist in such localities where repeated fecal pollution takes place.

In being confronted by the evidence of the high incidence of

infection by the gas bacillus in coal-mines, it was obvious that the bacterial contamination had been carried into the mine subsequent to its opening and development. The conditions that are present in the majority of these mines are favorable for the development and protection of bacteria which may find their way into the mine. The protection from light, drying, and excessive ranges of temperature permits the bacteria to persist for long periods of time in the damp soil.

In the examination of a coal-mine in which mules were used in the most distant underground workings, we had no difficulty in isolating *B. welchii* from the soft earth in several of the paths along which they traveled. It is probable that even when these animals are taken from a certain route the ground infection from the feces persists for a considerable period. Moreover, a certain amount of distant dissemination of the organisms takes place through the trickling of water and the carriage of the organism on the shoes of the laborers along many of the paths. It was found, too, that the men polluted the abandoned rooms or a portion of a room from which coal was being taken, by defecating in them. This practice leads to the distribution of large numbers of bacteria of all kinds to a region where there is a natural absence of organisms. Moreover, the injuries received by the miners by falling slate or coal were in the majority of instances received in the working rooms where this human pollution is found; while on the other hand, the injuries obtained about the truck cars were in the galleries where the mules are, or formerly were, frequently passing to and fro.

It seems quite obvious, therefore, that infection by *B. welchii* and other bacteria in coal-mines is a direct outcome of fecal pollution of the ground by man and animals.

It has been repeatedly noted at autopsy that much hemolysis occurred in individuals infected by the gas bacillus. The blood within the vessels is laked, and the vessels immediately after death, and probably during life, show a cherry-red staining of the tissues. Not only do the blood vessels show this hemoglobin imbibition, but it is also to be observed in the parenchymatous organs. Our observations on the hemolytic effect of our cultures on blood agar is in agreement with these vital findings.

More definite evidence of the effect of the gas bacillus upon the red blood cells is brought forward by the clinical blood examinations carried out under the direction of Dr. G. L. Hays. We are much indebted to Dr. Hays for the privilege of using some of his records of blood counts. It is quite obvious that, on account of the nature of this infection, there is quite a variation in the constitutional reaction in different individuals.

In some cases the infection is very localized, in others the emphysematous gangrene spreads progressively, while in the very severe cases the organisms soon become invaders of the blood stream. In like manner, the effect upon the blood-cells is directly dependent upon the absorption of the hemolysins from the infected site, the blood infection naturally being the most severe.

November 1, 1910......	Coal miner, adult, 39 years. Compound fracture of tibia by a coal car in the mine. Arrived at hospital seven and one-half hours after injury was received.		
November 3, 1910......	*B. welchii* was isolated		
	Red cells	3,450,000	Hemoglobin
	Leukocytes	17,800	
November 6, 1910......	Red cells	3,240,000	Hemoglobin 78 per cent
	Leukocytes	19,420	
November 7, 1910......	Red cells	3,190,000	Hemoglobin 78 per cent
	Leukocytes	20,400	
November 8, 1910......	Red cells	2,880,000	Hemoglobin 76 per cent
	Leukocytes	20,000	
November 9, 1910......	Red cells	2,170,000	Hemoglobin 73 per cent
	Leukocytes	15,120	
November 13, 1910......	Red cells	2,220,000	Hemoglobin 75 per cent
	Leukocytes	13,250	
November 15, 1910......	Red cells	2,280,000	Hemoglobin 75 per cent
	Leukocytes	11,200	
November 17, 1910......	Red cells	2,940,000	Hemoglobin 77 per cent
	Leukocytes	10,920	
November 19, 1910......	Red cells	3,130,000	Hemoglobin 77 per cent
	Leukocytes	9,800	
November 22, 1910......	Red cells	3,440,000	Hemoglobin 78 per cent
	Leukocytes	9,400	
November 26, 1910......	Red cells	3,840,000	Hemoglobin 81 per cent
	Leukocytes	8,000	
December 11, 1910......	Red cells	4,680,000	Hemoglobin 84 per cent
	Leukocytes	7,800	

Recovery

In two other cases blood counts were similarly carried out. In the one, the red cells fell to 2,590,000, in the other to 4,000,000. It was observed in each case that the greatest anemia was not immediately after the accident when a certain amount of hemorrhage occurred, but the fall in the red cells was progressive from day to day until the infection was overcome and convalescence was taking place. It is quite probable that this secondary anemia is an important factor in determining the issue of this infection. It is apparent, too, that much of the hemoglobin continues to circulate with the blood, the liver and the spleen being unable to remove the quantity liberated.

BACTERIOLOGICAL.

The isolation and determination of *B. welchii* from infected wounds is attended by considerable difficulties. The first and majority of the cases here reported were determined by the simple methods described by various authors, and which can be only taken to indicate the presence of one of the members of the gas-bacillus group. In the last nine cases attempts were made to isolate the organisms in pure culture and to determine the cultural characteristics of each. It has been our experience that these infected wounds always harbored mixed cultures. In one instance a pure culture of *B. welchii* was obtained from the heart blood at autopsy. The wound (compound fracture of the leg) contained several types of bacteria, including *B. welchii*, but the gas bacillus was the only one which invaded the blood. As this autopsy was performed one hour after death it is probable that the organism began its invasion of the systemic circulation during life. Streptococci are probably most frequently associated with the infection by the gas bacillus, and it is even suggested by some that *B. welchii* requires this or some other organism to produce conditions favorable for its growth in the tissues. Welch, however, has shown that aseptic lesions, presenting areas of less resistance, readily become the nidus for the gas bacillus, and that, therefore, a primary infection of a different nature was not essential. The nature of the bacteria present in a lacerated wound is obviously dependent upon the bacteria present

in the contaminating materials. Hence, the types of the organisms which may be associated with *B. welchii* are very numerous.

In the usual routine the determination of the infection in a case of gaseous emphysema has depended upon both a clinical and preliminary bacteriological examination. The presence of a lacerated wound, not in the region of the lungs or air passages, showing a progressive infiltration with gas, along with more or less edema and gangrene, from which the smears show large gram-positive bacilli with rounded ends and occasionally showing spores and capsules has, along with the cultural production of a "stormy fermentation" in milk, been indicative of a gas bacillus infection. To this, the presumptive test of gas production in the tissues of an animal which had been inoculated, killed, and incubated, has been of further confirmatory value.

It is evident from the reports of Klein that other organisms belonging to this group may produce gas in the tissues, so that the presumptive test by the inoculation of material into the tissues of an animal with subsequent gas production does not prove the identity of the organism but rather of a group of organisms.

It has been observed by others that some confusion has arisen on account of the varying staining reactions exhibited by this organism. We have noted that *B. welchii* stains readily with the simple stains, and that the majority of the organisms obtained from the infected tissues are gram positive. In such smears a few bacilli lose the gram stain. The organisms, however, taken from older cultures have a most irregular action to this stain. In some cases the majority may be gram negative while others contain gram-positive masses or show a polar staining. The older cultures tend to become gram negative, but fresh cultures from these may again give rise to gram-positive organisms. It was observed, however, that certain cultures had a greater tendency to produce irregular staining and gram-negative forms than others. In a like manner there is some difficulty in obtaining uniform results with the capsule stain. We have found that capsules are best demonstrated from the fresh tissue and from the growths on Loeffler's blood serum.

B. welchii is non-motile. Spores are not readily formed. We have observed them both in fresh smears from the tissues and also

in cultures on Loeffler's blood serum, but they are seldom present in abundance. In one strain isolated we have obtained spores in cultures with comparative ease.

In our later cases we have isolated *B. welchii* in pure culture and determined the cultural characters. These have corresponded to the characters described by Welch, and to them we have added a number of other features which may be of assistance.

For the production of anaerobism we have used the pyrogallic-acid method, while for the separation of mixed cultures we have used the deep-agar (dextrose) shake method. In almost all cases it was necessary to repeat the process for separation several times before an individual pure colony could be picked. This was then planted in media for the differentiation. It was observed that the complete fermentation of the various media occurred very rapidly and that no further change took place in the media after 48 hours. The inoculated gelatin was incubated with the other tubes, and after removal from the incubator it was chilled in the refrigerator. By this means growth was always obtained and the complete liquefaction of the gelatin occurred in 24 to 36 hours; when, however, the gelatin cultures were permitted to develop at lower temperatures, the growth and liquefaction were very much slower. We believe that if the method of incubation is used for gelatin cultures, uniform results of liquefaction of this medium will be obtained for this organism. The following characters and reactions on media were obtained when *B. welchii* was grown anaerobically:

Broth	Poor growth at bottom of tube. Upper liquid clear
Agar slant	Fine discrete translucent gray colonies of less than pin-head size
Blood agar	Fine discrete gray colonies with hemolysis
Gelatin	Complete liquefaction
Dunhams	No indol. Growth poor
Dextrose broth	Acid and gas
Lactose broth	Acid and gas
Saccharose broth	Acid and gas
Mannite broth	No acid and no gas
Potato	Invisible—sometimes a moist appearance along line of inoculation
Milk	Acid and coagulation with gas. "Stormy fermentation." Strong odor of butyric acid
Loeffler's blood serum	Moist gray colonies, sunken below the surface. Liquefaction of the medium continues

The fermentation of various sugars can also be observed in stab or shake agar to which the various sugars have been added. In such agar tubes the fermentation was at times so great as to force the plug and some of the agar out of the tube. The surface colonies were found to rapidly lose their vitality so that further transfers from them were unsuccessful. The inoculation of the organism into the blood stream of the small animals gave rise, after death, to a great production of gas in the tissues of the entire body. From these tissues the bacillus was again isolated.

In an attempt to do away with animals in these experiments, we introduced the bacillus into fresh liver tissue obtained from the butcher. Both beef and calf livers were used, and although a production of gas was obtained in them, we found an equal amount produced in the uninoculated controls. From these control portions of the liver, a gram-positive bacillus was obtained closely resembling *B. welchii*, but we have not up to the present been able to isolate it in pure culture. These microorganisms with the production of gas in beef and calf livers were obtained in six different controls and precluded the use of these tissues for these experiments. We similarly encountered difficulties in the use of guinea-pig liver. In the latter instance, some of the liver of a guinea-pig was removed sterilely and placed in the Smith fermentation tubes to facilitate the anaerobic condition. We found, however, that this too was impractical, in that there was a gas production in the uninoculated liver, with the growth of a gas-bacillus-like organism. These results are very similar to those of Wolbach and Saiki who found that the healthy livers of dogs contained bacilli growing only anaerobically.

From one of the human cases there was isolated an organism with the general characters of *B. welchii* and which by the simple methods would have been classed as this bacillus. It was found, however, to be quite distinct in its cultural characters from the one above described.

The organism was obtained from the lacerated wound of a compound fracture which ended fatally, and by means of anaerobic shake cultures it was separated in pure culture from streptococci and from gram-negative aerobes. The bacilli were large organisms

with blunt ends and rounded corners. They stained readily with ordinary stains and were gram positive. Spores were observed in the original material and were also seen later in old serum cultures. The organism usually appeared singly or in pairs, seldom in chains. Cultures were only obtained by anaerobic methods. Motility was observed in fresh cultures. The cultural characters were as follows:

Broth	Very scanty growth at bottom
Agar	Thin transparent grayish growth along streak
Gelatin	No liquefaction
Potato	Much gas in fluid below medium; surface of medium moist; colony invisible
Dunhams	No indol
Litmus milk	Acid with firm clotting, gas and clear whey, odor of butyric acid. No softening of clot
Dextrose broth	Acid and gas
Lactose broth	Acid and gas
Saccharose broth	Acid and gas
Mannite broth	No acid, no gas
Dulcite	No acid, no gas
Loeffler's blood serum	Fine gray colonies with gray flocculi in the water condensation. No evidence of liquefaction

Animal test.—The inoculation of the organisms into the circulation led to a great distension of the tissue and organs with gas (18 hours after the death of the animal).

Subcutaneous inoculation of the organism into the healthy tissues of a rat was without effect.

We have, then, to deal with an organism belonging to the gas-bacillus group, but differing from the bacillus of Welch in the presence of motility, the non-liquefaction of gelatin and blood serum, and the readiness with which it produced spores on serum media. Spores were also observed in the colonies of deep-agar shake cultures. The surface colonies on agar were more diffuse and not so discrete as those of the true *B. welchii.* It simulated closely *B. butyricus* of Botkin as described by Klein.

SUMMARY.

There appears, from our findings, to be an unusual frequency of infection by the gas bacillus among those receiving lacerated injuries in the coal-mines. This infection gives all the clinical manifestations of gaseous phlegmon while the bacteriological

analysis indicates the presence of a member of the *B. welchii* group of organisms. These organisms, save one, which we have been able to isolate in pure culture and cultivate on differential media, conform with the characters of the *B. welchii* which have been described by Welch. One strain of organism was isolated which had many of the characters of *B. butyricus* of Botkin.

In the light of Klein's work on *B. enteriditis sporogenes* and *B. butyricus* of Botkin and with our isolation of an organism belonging to the gas-bacillus group having, however, definite characters separating it from *B. welchii*, it is evident that other anaerobic bacteria, simulating in many respects the gas bacillus of Welch, may produce an emphysematous gangrene in man. In all probability, therefore, the infection by the so-called gas bacillus indicates a disease produced by a group of organisms with many features in common rather than by a single organism.

The clinical observations of the rapid destruction of the red blood cells during life in these infections is of great interest, and is probably associated with the rapidly fatal course in many cases. The hemolytic effect may also be observed in cultures.

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THE CLEANING EFFICIENCY OR SANITARY VALUE OF VACUUM CLEANERS.*

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Testing of vacuum cleaners.—Methods of cleaning have been tested in the past by determining the number of bacteria which are found in the air over and above those normally present. That is, they have been tested according to their ability to clean without raising a dust. One broom during the process of sweeping would introduce a certain number of bacteria into the air, another broom or process of sweeping would introduce only half as many and would therefore be considered twice as good. With the introduction of the vacuum cleaner this method is evidently insufficient to test its cleaning value. What one wants to determine here is the effectiveness of the apparatus in removing the bacteria from the floor or other article to be cleaned, for probably no vacuum cleaner, however inefficient, will raise much dust, although in this connection special attention is directed to the findings given below in regard to cleaners which exhaust into the air of the room being cleaned. What we want to know here is the proportion of dust and bacteria removed from the room by the cleaner. This is a difficult matter to test bacteriologically, and one which, so far as the writers know, has not been attempted before. None of the ordinary methods of bacteriological analysis are applicable. It seemed necessary, therefore, to devise a method. The problem, as it appeared to us, was to determine the number of bacteria on a given surface of floor or furniture; clean it and then determine the number of bacteria left. In this way it would be possible to determine the cleaning efficiency of a system measured in terms of bacteria removed.

The first method that suggested itself was to wipe a measured area of the surface to be cleaned with a sterile cotton swab moistened in sterile water, the cotton swab to be put into a flask of

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sterile water, used as the diluting medium. This idea was put into action and definite areas were marked out on the floor and the method tried out. Not one swab was used but a number consecutively until all the dirt appeared to be taken up. It was found impossible, however, to arrive at any definite conclusion as to the number of bacteria present, because of variations in different trials which apparently could not be overcome. While working at this problem, one of the writers had occasion to use some surgeon's adhesive tape and this suggested the idea that the principle involved here might be made use of in removing bacteria. Various adhesives were tried. The method which was finally agreed upon is as follows.

Discs of cheesecloth the size of a Petri dish are cut out, leaving a small protuberance on each of two sides. These protuberances are used later in raising the disc. The Petri dish, with its cloth disc, is sterilized in the hot-air oven. A thick gelatin is then prepared (20 gms. of gelatin to 100 c.c. water) and sterilized in the autoclave at 12 pounds pressure for 15 minutes. Special care must be taken to heat the autoclave rapidly and cool it down quickly, as it is imperative that the texture of the gelatin remain firm and tenacious. The gelatin is then quickly poured into the Petri dishes containing the cheesecloth and set away to harden in a cool place. Great care must be taken in handling the Petri dishes while the gelatin is still liquid since if it gets between the covers it will glue them together. The texture of the gelatin is at its best when 24 hours old.

Other materials needed for this work are flasks of sterile water, containing 50 c.c., 100 c.c., 200 c.c., and 1,000 c.c. The amount used will depend upon the condition of the surface to be tested. The flasks containing the sterile water should have wide mouths, such as Phillips beakers. Also sterile forceps, sterile pipettes, a sterile spatula, sterile Petri dishes, and a water bath should be at hand.

The adhesive disc should be carefully removed from a Petri dish with sterile forceps and then placed on the surface to be tested and pressed gently with a sterile spatula, so that the entire undersurface of the disc comes in contact with the surface to be tested.

The disc is then carefully picked up and placed in a sterile water blank. While doing this work great care must be taken so as not to handle the disc any more than is necessary. The water blanks are then placed in a bath, the water of which is not higher than 45° C. The discs will dissolve in about 15 minutes. When the gelatin is thoroughly dissolved the disc of cheesecloth will float in the water. Three plain agar plates are then poured, using one c.c. of the dilution in each. They are incubated at room tempera-

Fig. 1.—Apparatus Used to Test Vacuum Cleaners.

1. Petri dish with cheesecloth disc.
2. Gelatin disc in lower half of Petri dish.
3. Phillips beaker containing sterile water in which gelatin disc is being dissolved.
4 and 5. Sterile forceps and spatula.

ture for five days. When the colonies have developed, the number of bacteria per sq. cm. of the space tested is calculated.

Efficiency of adhesive discs in removing bacteria.—In order to prove that the adhesive discs take up the greater part of the bacteria, several discs were consecutively applied to the same area. When the first disc was plated out it was found that 22 bacteria per sq. cm. were taken up; when the second disc was applied to the same area, immediately afterward, less than one bacterium per sq. cm. was found. A third disc removed none per sq. cm. Two other tests made in a similar manner gave practically the same results.

The efficiency of the discs in removing bacteria was shown in another way. Black loam was sterilized in the oven and then pulverized in a mortar. This sterile, pulverized dirt then had mixed with it several loopfuls of *B. prodigiosus* taken from an agar culture. This dirt was sprinkled over a smooth surface, such as the top of a sterile Petri dish, and then the discs were applied to this, and dilutions and plates made in the ordinary way. An average of four tests showed that the first discs took up 7,166 bacteria per sq. cm., that the second discs found only 56 bacteria per sq. cm., and the third, less than one per sq. cm. It is thus seen that the first discs take up more than 99 per cent of the total number of bacteria on the surface, which is well within the limits of experimental error.

Number of bacteria on floors.—Having now worked out a method for removing bacteria and having tested the same, and having come to the conclusion that it removes fully 99 per cent of the bacteria on a surface, we may turn to a study of the number of bacteria found on ordinary surfaces and the efficiency of vacuum cleaning methods.

A study of the bacteria on a small area of a rather dirty laboratory floor showed 11,850 bacteria per sq. cm. In another place, the garret stairs of a dormitory, curiously enough, almost exactly the same number was found, i.e., 11,820. As an average of a number of trials in an ordinary room, 1,166 were found. At another time, on a stairway which had recently been cleaned, 68 bacteria were found as an average of a number of tests. In a recitation room 331 bacteria were found as an average of six tests, and 91 as an average of two tests in another room. In an exceptionally clean house, where a vacuum cleaner had been used for some months, an average of two different tests showed 84 bacteria per sq. cm.

VACUUM CLEANERS.

General description and types used.—When the vacuum cleaning system was introduced a few years ago, it was quite generally believed that the sanitary cleaning of buildings could be readily accomplished. But in the last few years there have been so many

different makes put upon the market, that vary greatly in their mechanical efficiency, that it would seem desirable to have some criterion for judging of their efficiency from a sanitary standpoint. If they differ greatly in mechanical efficiency they must also differ in cleaning power, and it was our purpose to test some of them. We have chosen two permanent systems, one in which the vacuum is produced by an electric fan, and the other by means of a steam aspirator. Of the portable types eight have been tested, three where the vacuum is produced by rapidly revolving fans, and two by bellows electrically driven, and two worked by hand. The experimental data on these different machines will be discussed separately.

Tests of Type A.—In Machine A the vacuum is obtained by an electric fan. This machine is installed in a women's dormitory and had been running about a year when the tests were made. The results obtained by an examination of the dirt on the floor before and after sweeping with the machine, together with the percentage of the efficiency, are shown in Table 1. This machine was further tested by means of the *B. prodigiosus* dirt and the results of this are shown in Table 2.

TABLE 1.

Tests Made on a Permanent System, Vacuum Obtained by a Fan (Bacteria per sq. cm.).

No. Exp.	Place	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
1	Dirty stairs	5,277	30	
2		5,072	46	
3		1,477	866	
Average		3,942	314	92 per cent
4	Well kept floor	33	0	
5		23	1	
6		55	5	
7		3,321*	7	
8		45	13	
9		23	2	
Average		1,166	9	99 per cent
10	Clean floor	25	4	
11		42	2	
12		28	0	
13		52	0	
14		58	2	
Average		68	3	86 per cent

* This large count is due to the presence of a piece of dirt.

In Table 1 tests were made under various conditions. In some cases the floors were very dirty; in other cases, very clean, and this probably accounts for the variation in efficiency. In experiments 1, 2, and 3, the floor was very dirty, and the bacteria were not easily dislodged by an air current, but were pulled off by the adhesive disc. In experiments 4 to 9, which were made on a very clean part of the floor, washed several times a week, the highest efficiency was obtained.

The tests with *B. prodigiosus* dirt (Table 2) show what this system can do when the material is not too firmly attached to the floor; and in the case of this machine practically all of the organisms were removed.

TABLE 2.

Tests Made in the Permanent System, Vacuum Obtained by a Fan (Bacteria per sq. cm.), Using *B. prodigiosus*.

No. Exp.	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
1	28,688	43	
2	29,963	26	
Average	29,325	35	99.88 per cent

Tests of Type B.—The vacuum in this case is produced by a steam aspirator. It is a single sweeper plant installed in a large gymnasium. The results obtained by an examination of the floor before and after cleaning are shown in Table 3, and the results of the *B. prodigiosus* tests are shown in Table 4.

TABLE 3.

Tests Made on a Permanent System, Vacuum Obtained by Steam Aspirator (Bacteria per sq. cm.).

No. Exp.	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
1	110	17	
2	82	21	
3	33	5	
4	43	22	
5	83	8	
Average	117	24	79 per cent
6	19,600	7,000	
7	27,900	8,550	
8	35,700	8,250	
Average	27,700	7,933	72 per cent

TABLE 4.

TESTS MADE ON A PERMANENT SYSTEM, VACUUM OBTAINED BY STEAM ASPIRATOR (BACTERIA PER SQ. CM.), USING *B. prodigiosus.*

No. Exp.	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
1	1,500	32	99.05 per cent
2	112,600	13,900	87.00 per cent

The first five tests were on a very clean floor; the last three on a very dirty one. The efficiency in both cases is about the same. It will be noticed that the percentage is considerably lower than with the previous type, due probably to the fact that the first machine moves a larger volume of air.

Tests of Type C.—Portable. Vacuum obtained by means of a rapidly revolving fan run by an electric motor. The testing was done in a recitation room, and the results obtained are shown in Table 5.

TABLE 5.

TESTS MADE ON PORTABLE MACHINE, VACUUM OBTAINED BY A FAN (BACTERIA PER SQ. CM.).

No. Exp.	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
1	75	5	
2	35	1	
3	844	298	
4	508	73	
5	377	44	
6	154	97	
Average	331	86	74 per cent

Tests of Type D.—Fan type, electrically driven.

TABLE 6.

TESTS MADE ON PORTABLE MACHINE, VACUUM OBTAINED BY A FAN (BACTERIA PER SQ. CM.).

No. Exp.	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
1	92	25	
2	48	22	
3	201	10	
Average	113	19	84 per cent

Tests of Type E.—Small portable machine, vacuum obtained by electric fan. Data shown in Table 7.

TABLE 7.

TESTS MADE ON PORTABLE MACHINE, VACUUM OBTAINED BY A FAN (BACTERIA PER SQ. CM.).

No. Exp.	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
1	57	5	
2	125	15	
Average	91	10	89 per cent

Tests of Type F.—Vacuum produced in these machines by means of a bellows and electric power.

TABLE 8.

TESTS MADE ON PORTABLE MACHINE, VACUUM OBTAINED BY BELLOWS (BACTERIA PER SQ. CM.).

	No. Exp.	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
Type F. Machine 1	1	38	17	
	2	130	21	
	Average	84	19	77 per cent
Type F. Machine 2	3	45	25	
	4	122	12	
	Average	83	18	77 per cent

Tests of Type G.—A single machine of this type was tested. In it the vacuum was obtained by means of a bellows (hand driven).

TABLE 9.

TESTS MADE ON PORTABLE MACHINE, VACUUM OBTAINED BY BELLOWS (HAND DRIVEN) (BACTERIA PER SQ. CM.).

No. Exp.	No. Bacteria Found on Floor	No. Bacteria after Cleaning	Percentage of Bacteria Taken Up in Cleaning
1	11	6	
2	10	3	
Average	10	5	57 per cent

The efficiency of the permanently installed systems is much higher than in the case of the portable machines. Judged by the number of bacteria which they remove from the floor, the efficiency is 92 (A) and 75 (B) per cent as compared with 74 (C), 84 (D),

89 (E), 77 (F), 57 (G) per cent. The efficiency of the portables varies greatly, due to the differences in motive power, manner of construction, shape and size of brushes, etc.

Discharge of bacteria into air through exhaust pipe of machine.—In the permanently installed systems the bacteria in the dirt are taken out of the rooms entirely, and if the discharge pipe is properly located the bacteria removed are of no concern. In the portables, however, the condition is very different. All of these examined so far have discharged the air directly into the room. In some of them the air has been filtered or strained through a bag; in others the dust and bacteria are retained by a series of baffles. It is very evident upon consideration that it is ordinarily quite impossible to keep the bacteria from getting back into the room. In some of the machines examined, the bacteria pass through very readily; in others it is with greater difficulty, apparently, that their passage is accomplished. The testing of this point has been carried out by means of placing culture plates in front of the discharge pipe, and also by sucking dirt containing *B. prodigiosus* into the machine and then determining whether or not it escapes from the machine, and if so, the distance to which it is thrown, etc. The experiments were carried out as follows:

Dirt containing *B. prodigiosus* was sprinkled on the floor and sucked up in the machine to be tested. The machine was then taken into an entirely separate room where the number of bacteria in the air had just been determined. The machine was then set in motion and culture plates were exposed at various distances from the discharge pipe. The total number of bacteria on these plates were then determined, as well as *B. prodigiosus*. The results obtained are shown in the accompanying diagram (Fig. 2). This shows that a very considerable number of bacteria are thrown out of these machines and that some of them are thrown to a distance of nearly six feet.

It will be seen from the experiments that from a sanitary standpoint there is a great difference between the various types of machines. In some cases the bacteria are thrown back into the room in considerable numbers and for some distance. These

machines are able to take up the dirt and bacteria in varying degrees of perfectness, but since they allow the escape of the bacteria contained in the dirt they may be an actual menace to health. And it is possible to obtain abundant data to warrant health officers forbidding the use of these machines by traveling cleaners, since it must be very evident that if *B. prodigiosus* can be carried from one room to another, *B. tuberculosis* could be carried from one house to another.

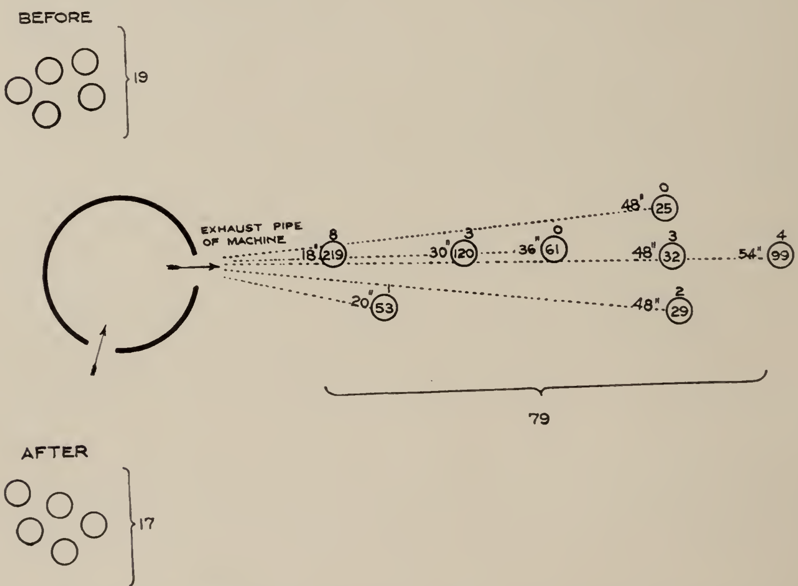

Fig. 2.—The large circle represents the vacuum cleaner; the small circles the culture plates; the dotted lines, the distances which are expressed in inches. The numbers inside of the small circles are the number of colonies of bacteria which developed. The figures just above the circles are the number of *B. prodigiosus* colonies. The figures on the brackets represent averages.

CONCLUSIONS.

1. Gelatin adhesive discs offer a satisfactory means of determining the number of bacteria on various surfaces, such as floors, furniture, etc.

2. Their use makes it possible to determine the cleaning efficiency of vacuum cleaners.

3. The permanently installed systems are most efficient.

4. The portables vary greatly in cleaning efficiency, some being quite efficient. Others have little cleaning power.

5. The portable machines, all of which allow the escape of the bacteria with the exhaust air, are of questionable value from a sanitary standpoint and under certain conditions they may be actually dangerous.

SUSCEPTIBILITY OF CERTAIN DOMESTIC ANIMALS TO PLAGUE INFECTION WITH PARTICULAR REFERENCE TO THAT OF GROUND SQUIRREL ORIGIN.*

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and
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The fact that frequently plague-infected ground squirrels have been found in pastures in California raised the old question of the susceptibility of farm animals to infection with *B. pestis*. It also seemed desirable to carry out experiments to determine the pathogenicity for domestic animals of the plague bacillus of ground squirrel origin. The work was undertaken at the suggestion of Surgeon Rupert Blue, United States Public Health and Marine Hospital Service.

The subject has been investigated by several observers with reference to the plague bacillus from man and from rats. Unfortunately some of the original communications are not now available to us.

Simpson[1] gives a review of the subject in connection with the report of his own work, and from it we glean the following data. In the tabulated results of the investigations of the German and Austrian commissions it appears that constitutional and local reactions were observed in a considerable number of cases. Disregarding intravenous and intraperitoneal inoculations, there appeared to have been no deaths proven to be due to plague among animals other than rodents and anthropoids, with the exception of some dogs and cats reported by the Austrian Commission.

We quote from Simpson's review, giving the results of experiments of certain other workers: "Haffkine experimented on horses, cows, sheep, and goats by inoculation of plague cultures, but the goats alone, without developing any acute disease, lost condition gradually, wasted away, and after a considerable time many of them succumbed. Lowson experimented on pigeons, ducks, crossbills, yellow-hammers, linnets and canaries, and failed to infect them with plague. On the other hand, Wilm, in the Hongkong epidemic of plague in 1896, succeeded in infecting a pig fed with the spleen of a man who had died of plague; and a number of poultry fed by

* Received for publication August 29, 1911.

[1] *A Treatise on Plague*, The University Press, Cambridge, 1905.

him with plague material and with pure culture of the plague bacillus died in three or four days of plague. Piaxi and Posen found that when pigeons and sparrows were starved they were susceptible to plague."

The following statements are from Simpson's account of his own work. "The result of these experiments was to establish the fact that calves, hens, turkeys, geese, pigeons, sheep, and pigs were susceptible to plague both by inoculation and by feeding, and that pigs and poultry were susceptible in a high degree.

"Of the 15 pigs experimented on 13, equal to to 86 per cent, died; of the eight calves seven, equal to 87 per cent, died; of the 31 hens 11, equal to 35 per cent, died; of the seven pigeons all died; of the six geese three, equal to 50 per cent, died; of the six turkeys four, equal to 66 per cent, died; of the six ducks all died; of the three red-beaks two died; of the seven monkeys five, equal to 70 per cent, died; of the seven guinea-pigs all died; of the 109 rats 72 per cent died."

Bannerman and Kapadia[1] analyze Simpson's observation and express themselves as not convinced from the data given that all of his animals died of plague. The following statements are condensed from their review: Lieut. Walton, I.M.S., failed to infect pigeons by hypodermic injection. London made extensive experiments in Russia with the birds of that country both in a normal state and after depression of vitality by starving, chilling, etc., and concludes that birds are not susceptible to plague. Hill, in Natal, attempted to infect domestic animals by feeding, inoculation, etc. Pigs and calves were used without result, except "transient illness due to manipulation" in the case of one of the pigs.

Bannerman and Kapadia experimented with the following animals: four pigs, four turkeys, two calves, four geese, four fowl, and four ducks. The methods of feeding, scarification, and subcutaneous inoculation were employed. One pig (scarified) was temporarily indisposed; one pig (subcutaneous inoculation) was likewise indisposed and developed a local abscess. Otherwise, the series was negative.

DeSouza, Aruda, and Pinto[2] experimented with the following animals: Two calves, eight pigs, six dogs, 10 rabbits, two ferrets, one goat, eight pigeons, one turkey, 85 chickens, and two cats. Aside from a few local and temporary reactions, and deaths not proven to be due to plague, their positive results comprise the 10 rabbits, one ferret inoculated by the subcutaneous method, and one cat infected by feeding. These authors also give a preliminary report of 23 naturally infected cats. Most of these had cervical buboes and were probably infected by feeding. There were also cases of axillary and inguinal buboes and one popliteal, and the authors express the opinion that these animals were infected by fleas; two succumbed to primary pneumonic plague.

McCoy[3] working with a strain of plague originally isolated from a ground squirrel obtained the following results: Two dogs and two pigeons inoculated subcutaneously were negative; one cat inoculated intraperitoneally, four inoculated subcutaneously, and two out of four inoculated by scarification, died of plague.

In our experiments we employed, in one series of inoculations, a culture (squirrel 2010) which had been isolated a few days previously from a guinea-pig dead of acute plague after cutaneous

[1] *Jour. of Hyg.*, 1908, 8, p. 209. [2] *Jour. of Hyg.*, 1910, 10, p. 196.

[3] *Pub. Health Bull. 43*, Pub. Health and Marine Hosp. Serv., Washington, D.C.

inoculation from a naturally infected ground squirrel. In this experiment we used one pig, one calf, and one goat, all of which had survived inoculation with the plague-like disease[1] of rodents. Each animal was given one 72-hour agar culture subcutaneously. The pig and the calf showed merely a temporary indisposition. The goat was not made ill. Sixty days after the inoculation all of the animals were in good health. The guinea-pig and the white rat controls vaccinated with the same culture died of plague on the sixth and fourth days respectively.

In the remainder of our work the strain employed (squirrel 881) had been carried for some months on artificial media. This culture was known to be virulent. It had been employed in other experimental work, including an investigation of the potency of commercial samples of anti-pest serum.

Five grown cats which had proven negative to the plague-like disease were vaccinated with a strong emulsion of a 48-hour agar culture fourth generation. A guinea-pig and a white rat were used as controls. The results are shown in the following table.

TABLE 1.

Animal	Day of Death	Result
Cat A..........	8th	Emaciated. Tubercle-like granules in liver and lungs. Guinea-pig inoculated cutaneously from liver of this cat died on 8th day with the lesions of plague
Cat B..........	9th	Necrotic area surrounded by gelatinous infiltration at site; caseous right inguinal and axillary buboes; large, dark spleen; characteristic bacilli in smears from bubo
Cat C..........	....	Killed on 17th day; no lesions
Cat D..........	....	Killed on 17th day; small necrotic gland in groin
Cat E..........	....	Killed on 17th day; necrotic gland in groin. Guinea-pig inoculated cutaneously from this gland died on 5th day with typical lesions of plague
Guinea-pig (control)	8th	Subacute plague
White rat (control)..........	4th	Acute plague

In another series we used one pig, one calf, one goat, and one sheep. Each animal was given a 48-hour agar culture subcutaneously. Two frogs and one pigeon were each given two loops of the growth. A guinea-pig and a white rat which served as controls were vaccinated with the same culture. The guinea-pig died on the twelfth day and the white rat on the fifth day. Both animals had the gross and the microscopical appearances of plague.

[1] There is no reason for believing that this disease immunizes against plague.

The calf and the pig showed evidences of slight illness for a day or two following the inoculation. The other animals remained well. All were kept under observation for three months, during which time they showed no symptoms attributable to the inoculation, other than those mentioned.

To determine whether the plague bacillus persisted in a virulent state in the bodies of the large animals, guinea-pigs or white rats were inoculated subcutaneously with material aspirated from time to time from the site of inoculation of the calf, hog, sheep, and goat of this series. A large hypodermic syringe was loaded with about 1 c.c. of physiological salt solution. This was injected into the tissue at the site and immediately sucked back into the syringe, and the needle was withdrawn. The material was used to inoculate the test animals—guinea-pig or white rat as the case might be. The diagnosis of plague in the test animals was usually made upon gross and microscopical appearances. In a few doubtful cases, cultures were made. In Table 2 the figures denote that the test animals died of plague on that day after inoculation. The minus sign indicates a negative result; that is, the animal survived or died from other causes, or was killed and examined after the usual period with negative results.

TABLE 2.

Animals Inoculated with Material Aspirated on Various Days.

	1st Day 24 Hrs. Guinea-Pig	2d Day White Rat	3d Day White Rat	4th Day White Rat	5th Day White Rat	7th Day Guinea-Pig	9th Day Guinea-Pig	12th Day Guinea-Pig	15th Day Guinea-Pig
Calf	3	2	9	—	—	5	5	—	—
Hog	3	5	3	3	3	8	—	*	—
Sheep	—	—	—	—	3	10	—	—	—
Goat	—	—	—	—	—	—	—	—	—

* Guinea-pig killed on the seventh day presented the usual lesions of subacute plague.

We are unable to account for the very evident discrepancies in the results of these inoculations. As a glance at the table shows, the goat is the only animal in which we failed to demonstrate the organism at some time in the aspirated material.

To determine the presence of antibodies in the serum of the goat, the calf, the pig, and the sheep of this series, blood was drawn from the animals on the thirty-seventh day after inoculation.

The clot was allowed to separate, and 5 c.c. of the serum was used to inject white rats subcutaneously 24 hours after the bleeding. For controls, serum was used from defibrinated blood obtained from the slaughter-house from a cow, a hog, and a sheep. No normal goat serum was available. The animals were then vaccinated with a two-day old culture of *B. pestis* of squirrel origin (No. 2010). The results are shown in Table 3.

TABLE 3.

Weight of White Rat, Grams	Serum from	Day of Death	Lesions	Remarks
65	Goat	....	None	Killed 11th day
120	Hog	....	"	Killed 11th day
240	Calf	10th	Subacute plague	
195	Sheep	8th	" "	
65	Control, no serum	4th	Acute plague	
145	Control, hog serum	3d	" "	
280	Control, calf serum	5th	" "	
230	Control, sheep serum	3d	Lungs consolidated	Cause of death not determined, probably not plague

To secure further evidence as to the protective power of the sera, white rats were given subcutaneously varying doses of the serum of the goat and of the calf of the above series. One control received a dose of normal calf serum, the other controls received no serum. These sera had been preserved for 12 days on ice with the addition of 1 per cent trikresol. The rats were then vaccinated with the bubo of a guinea-pig dead on the fourth day after inoculation from a naturally infected plague squirrel. The results are shown in Table 4.

TABLE 4.

Source of Serum	Dose	Weight of Rat, Grams	Day of Death	Lesions	Remarks
Goat	3 c.c.	90	6th	Acute plague	
Goat	2.5 c.c.	90	....	None	Killed 10th day
Goat	1 c.c.	90	....	"	Killed 10th day
Calf	3 c.c.	85	....	Caseous bubo	Killed 10th day
Calf	2.5 c.c.	95	....	Caseous foci in spleen	Killed 10th day
Calf	2.5 c.c.	135	5th	Acute plague	......
Calf	1 c.c.	110	4th	" "	......
Calf	1 c.c.	105	6th	" "	......
Calf	0.1 c.c.	100	3d	" "	......
Calf	0.1 c.c.	140	4th	" "	......
Control calf serum	2.5 c.c.	140	6th	" "	......
Control calf serum	none	130	3d	" "	......
Control calf serum	"	130	4th	" "	......
Control calf serum	"	135	4th	" "	......

The controls were all larger than the test animals and would have been expected to live longer than the latter, but as the table shows, they all succumbed to acute plague, while several of the smaller (protected) animals survived. Small doses of the calf serum exerted no protective influence.

An experiment was performed for the purpose of ascertaining whether normal calf serum plus 1 per cent trikresol had any influence on the course of plague in vaccinated white rats. The results showed that the serum-trikresol mixture was without protective properties.

We believe that these experiments indicate that the serum of the calf and that of the goat exerted decided protective action against virulent plague bacilli. Apparently the sheep and the hog serum also acted in this manner, but as only one test animal was used in each case no definite statement can be made.

SUMMARY.

Although there are a few discordant reports, the general experience with animals other than rodents and anthropoids seems to be that local and temporary constitutional effects are observed in a considerable number of cases after feeding or after subcutaneous inoculation with cultures of *B. pestis* or with plague tissues, but that fatal infections are extremely rare. The cat is an exception to this rule, and exhibits a considerable degree of susceptibility. The results of our experiments have been quite in harmony with the above generalization.

B. pestis was demonstrated at the site of inoculation after several days in the case of the calf, hog, and sheep, but not in the case of the goat.

Adequate doses of the serum of the goat and the calf inoculated with a culture of *B. pestis* certainly protected rats against plague infection. In the case of the hog and the sheep some protection was probably exerted.

ON THE NATURE OF THE PROTEOLYTIC SUBSTANCES IN THE BLOOD.*

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The extensive investigation of the phenomenon of anaphylaxis has brought out the importance of parenteral protein digestion in relation to both anaphylaxis and other processes of immunity.

Vaughan,[1] in 1906, advanced the idea that anaphylactic shock is caused by an intoxication with protein-split products, and that the condition of hypersusceptibility is due to an increase in the protein-splitting power of the blood. Since the work of Vaughan a mass of evidence in support of this view has developed. The toxicity of protein-split products has been shown by Vaughan and Wheeler,[2] and Biedl and Kraus.[3] The production of protein-split products by the action of the serum of hypersensitive animals on the sensitizing antigen has been demonstrated by Pfeiffer and Mita,[4] and Rosenow.[5] Recently Friedberger and his associates,[6] Neufeld and Dold,[7] and Rosenow,[8] have demonstrated the production of substances of the nature of protein-split products by the action of immune serum on bacterial antigen. Vaughan, Cummings, and Wright[9] have produced continuous fever by repeated injections of small quantities of protein substances such as egg white. The parenteral digestion of bacterial proteins assumes therefore an important rôle in the production of the intoxications associated particularly with those organisms producing the so-called endotoxins.

It is obviously desirable to see whether the proteolytic ferments of the blood resemble the immune bodies. In order to study the specificity of these ferments, Abderhalden and Pincussohn[10] injected gelatin into dogs, and found that the ferments which subsequently developed split not only gelatin but also peptones from silk. With Immisch,[11] Abderhalden found that, in a similar way, ferments following injections of silk peptone split peptones from silk and edestin. With Israel[12] he obtained a similar result with edestin; that is, the ferments acted upon peptones from various sources. Abderhalden and Sleeswyk[13] found that ferments, developing after casein injections, acted on peptones from various sources. The conclusion drawn is that the ferments developing after these injections of proteins were not specific.

Fleischmann[14] was able to obtain a precipitin for heterologous proteins by injecting into animals, split products of proteins. According to Fleischmann, and also

* Received for publication September 1, 1911.

[1] *Jour. Am. M. Ass.*, 1906, 47, p. 1009.

[2] *Jour. Infect. Dis.*, 1907. 4, p. 476.

[3] *Wien. klin. Wchnschr.*, 1909, 11, p. 363.

[4] *Ztschr. f. Immunitätsf.*, 1910, 6, p. 18.

[5] *Jour. Infect. Dis.*, 1911, 8, p. 190.

[6] *Deut. med. Wchnschr.*, 1911, 37, p. 377; *Ztschr. f. Immunitätsf.*, 1911, 9, p. 369.

[7] *Berl. klin. Wchnschr.*, 1911, 48, p. 1069.

[8] *Loc. cit.*

[9] *Ztschr. f. Immunitätsf.*, 1911, 9, p. 458.

[10] *Ztschr. f. phys. Chem.*, 1910, 64, p. 100.

[11] *Ztschr. f. phys. Chem.*, 1910, 64, p. 423.

[12] *Ibid.*, p. 425.

[13] *Ibid.*, p. 427.

[14] *Ztschr. f. klin. Med.*, 1906, 59, p. 515.

Obermayer and Pick,[1] the grouping upon which protein specificity depends is destroyed by the digestion of the protein. Gelatin, according to the work of Wells,[2] might also be expected to give rise to substances acting upon heterologous proteins, possibly on account of the relative freedom from aromatic radicals.

It seemed desirable therefore to study the specificity of ferments developing after the injection of complex proteins. Accordingly a rabbit was injected subcutaneously at four- to six-day intervals with 2, 4, 6, and 10 c.c. of sheep serum. In order to avoid the complicating action of precipitins the serum was tested five weeks after the last injection, as at that time it was found that the precipitins had disappeared. The optical method of Abderhalden was used:[3] 2 c.c. of the rabbit serum were mixed with 3 c.c. of sheep serum which was heated to 56° C. for one-half hour. The mixture was diluted 1–6 with salt solution and its optical activity immediately determined by means of the polariscope, a 10 cm. tube being used. The remainder of the mixture was then incubated for 24 hours at 37° C. and its optical activity again determined. All possible precaution was used to prevent bacterial contamination. A similar experiment was carried on at the same time, using the same rabbit serum but substituting bovine serum for the sheep serum. The polariscope readings follow:

	Sheep Serum	Beef Serum
Before incubation	0.67°	0.71°
After incubation	0.44°	0.69°
Change in angle of rotation	0.23°	0.02°

It will be seen that a change of 0.23° was found to follow the action of the rabbit serum on the sheep serum while a change of only 0.02° followed the action of the rabbit serum on beef serum.

In order to test further the specificity of the proteolytic ferments of the blood, another rabbit was immunized by repeated increasing doses of suspensions of typhoid bacilli, until four agar slants were given. After five weeks the serum was tested as follows: An extract of typhoid bacilli was made by growing large quantities of typhoid bacilli on agar in Roux flasks and washing them off in

[1] *Wien. klin. Wchnschr.*, 1906, 14, p. 327.

[2] *Jour. Infect. Dis.*, 1908, 5, p. 449.

[3] *Loc. cit.*

distilled water. The bacilli were then dried in a vacuum desiccator at 55° C., ground with sterile sand, and extracted with salt solution. The extract was filtered through a porcelain filter and heated to 60° C. for one hour. In a similar way an extract of colon bacillus was made and diluted to a strength giving nearly the same degree of optical rotation as the solution of protein from typhoid bacilli. To 16 c.c. of typhoid extract 4 c.c. of sheep serum was added, and the reading taken at once with the polariscope. The mixture was incubated for 24 hours and a second reading made with the remaining 10 c.c. Mixtures of colon-bacillus extract and rabbit serum were made in the same way, using the same rabbit serum that was used with the typhoid extract. The reading follows:

	Typhoid Extract	Colon B. Extract
Before incubation	1.05°	1.10°
After incubation	0.70°	0.99°
Difference in angle of rotation	0.35°	0.02°

It will be seen that there was a change of 0.35° in the typhoid extract, whereas in the colon-bacillus-extract mixture a change of 0.02° occurred. It is evident that in both the case of the serum and bacterial extracts the action of the ferments was much more marked in the case of the homologous protein than in that of the heterologous.

The action of normal rabbit serum and that of the serum of the rabbit immunized to sheep serum were compared. Two mixtures were made, using in one 2 c.c. of normal rabbit serum and 3 c.c. of sheep serum heated to 60° C. for one-half hour, and in the other 2 c.c. of immune serum and 3 c.c. of heated sheep serum. The readings of the mixtures before and after incubation (dilution of mixture 1–6) follow:

	Immune Serum	Normal Serum
Before incubation	0.72°	0.70°
After 24-hour incubation	0.44°	0.56°
Change in angle of rotation	0.28°	0.14°

A similar experiment was made substituting, instead of normal serum, immune serum which had been heated to 56° C. for one-half hour. The results are as follows (dilution 1–5):

	Active Serum	Heated Serum
Before incubation	0.86°	0.96°
After 24-hour incubation	0.32°	0.87°
Change in angle of rotation	0.54°	0.09°

It will be seen that there was a considerable change in the angle of rotation in the case of the normal serum (0.14°), twice as much with the immune serum, and very little (0.09°) with the heated serum. The serum used for the comparison of the active and heated serum was rich in precipitin, and it is probable that 0.09° difference in rotation both in the heated and unheated serum is due to simple precipitation of optically active protein.

In order to find out whether the heated inactivated serum could be reactivated or not as in the case of the lysins, Gruber[1] immunized animals to serum proteins and tested their action on silk peptone, using the optical method. He failed to obtain any reactivation. We must assume that any specific ferments comparable to amboceptors are concerned only in the splitting of the protein molecule to a point where the specific grouping is destroyed. In order to further digest these split products an excess of non-specific protein-splitting substances might be assumed. These ferments would be comparable to complement which is non-specific and cannot be reactivated. It is possible, therefore, that Gruber, in using silk peptone as an indicator, was attempting to reactivate only the non-specific ferments of Abderhalden.

The following attempt was made to reactivate a specifically proteolytic serum. Three mixtures were made:

Mixture 1: Heated immune serum 1 c.c., complement (guinea-pig serum) 1 c.c., sheep serum 3 c.c.

Mixture 2: Heated immune serum 1 c.c., salt solution 1 c.c., sheep serum 3 c.c.

[1] *Ztschr. f. Immunitätsf.*, 1910, 7, p. 762.

Mixture 3: Salt solution 1 c.c., complement 1 c.c., sheep serum 1 c.c.

The following are the readings of dilutions 1–6 of the mixtures, before and after 24-hour incubation:

	Mixture 1	Mixture 2	Mixture 3
Before incubation	0.62°	0.60°	0.53°
After incubation	0.34°	0.58°	0.39°
Change in angle of rotation	0.28°	0.02°	0.14°

The difference of 0.14° between Mixtures 1 and 3 can hardly be due to an additive effect, because the heated serum alone was almost without influence. It would appear to be rather in the nature of a reactivation.

CONCLUSIONS.

The results of these experiments would indicate that the proteolytic substances of the blood are of a nature resembling that of the immune bodies, especially the lysins.

THE COMPARATIVE VIRULENCE OF THE PNEUMOCOCCUS IN THE SPUTUM OF LOBAR PNEUMONIA AT VARIOUS STAGES OF THE DISEASE, WITH SPECIAL REFERENCE TO CRISIS.*

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There is no acute infection in which the clinical manifestations are more remarkable than those in pneumonia, and of these manifestations a typical crisis is the most striking. With the abrupt drop of temperature, pulse, and respiration, the patient passes from a "state of extreme hazard and distress to one of safety and comfort"[1] without any demonstrable change in the local condition. In solving the problem as to what happens at the time of crisis to produce this phenomenon, we must consider not only the methods of defense which the body adopts to get rid of the infection, but also the invading parasite. With the exception of a slight increase in agglutinins, the only difference so far determined between normal serum and the serum of immunized animals is in the presence of bacteriotropic substances which cause phagocytosis of virulent strains of pneumococci, while normal serum causes little or no phagocytosis.

G. and F. Klemperer, Neufeld, and others have also demonstrated protective substances in the serum of patients convalescing from pneumonia. In these sera, however, the immunity conferred is relatively slight, and they do not cause phagocytosis of virulent strains.

In the winter of 1909 I repeated the work of Graham on the phagocytability of the pneumococcus isolated from the sputum at various stages of the pneumonic process, using normal human serum and leukocytes. Forty hospital cases were studied. It was then noted that while the pneumococcus isolated before crisis was not phagocyted, the strains isolated at the time of crisis and

* Received for publication June 27, 1911.

[1] Osler, *Practice of Medicine.*

after crisis were actively phagocyted. This indicated some change in the organism other than a bacteriotropic one. This observation suggested the possibility of a virulence crisis at the time of the temperature crisis, since it had been generally established that the degree of phagocytosis of a given strain was a relative index of its virulence.

This problem has interested many investigators, and I shall attempt to give a rather full historical review because their results have been so vague and conflicting due to the various methods employed, and hence shall lay especial stress on the mode of isolation, amount injected, site of inoculation, weight of animal, and results obtained.

HISTORICAL.

With the discovery of the pneumococcus as the etiological factor of croupous pneumonia, various observers at first attempted to show the relative virulence of the pneumococcus from the various sources, in health and in disease, and later, to establish a virulence curve during the course of lobar pneumonia.

Fränkel isolated the pneumococcus by injecting one loopful of sputum[1] subcutaneously. After establishing the fact that he could demonstrate the pneumococcus for several weeks after crisis, he concludes as follows: "It seems to me that the virulence of the sputum after the crisis is not so high. During the active process, the rabbits died within 24 hours, while after crisis death was delayed several days, often until the sixth day."

Netter asserts that the expectoration during the first weeks after crisis is less virulent than before, that shortly after convalescence the saliva loses all virulence, but later regains its full pathogenicity.

Patella obtained lung juice at different stages of pneumonia by means of a hypodermic syringe. His results agree with those of Netter, that the pneumococcus gradually loses its virulence during the development of the pneumonic process.

Welch does not think that we are justified in laying down such definite laws regarding the loss of virulence as those made by Patella and Netter. He states that he has obtained from pneu-

[1] Personal communication to Dr. Stuertz.

monia at the time of crisis, and during recovery, pneumococci which were as virulent and sometimes more virulent than those procured from earlier stages in the same case.[1]

Kruse and Pansini isolated the pneumococcus at various stages of pneumonia by injecting 1 to 4 c.c. of sputum subcutaneously into rabbits. If the animal died with a septicemia, a broth culture was made from the blood; if, however, death occurred late, as a result of a local affection, the organisms were first isolated by the plate method and then transferred to broth. These broth cultures were placed in the thermostat for 24 hours, and 1 to 2 c.c. of the resultant growth were injected subcutaneously. Their conclusion is that the pneumococcus does not suffer a loss of virulence in the course of the pneumonic process. They were also of the opinion that the variation of virulence, determined by the inoculation of the original material from various sources, was probably due to a variation of the number of organisms injected, and also to the fact that many of the bacteria may have been dead.

Eyre and Washbourn isolated the pneumococcus by injecting the sputum into mice. After the death of the animals, cultures were made from the blood on blood agar tubes. A special loop containing about 0.5 gm. was used as a measure for subsequent inoculations into mice. The rusty sputum of two cases of pneumonia and the saliva of one normal case were studied.

Case 1.—Rusty sputum from a case of pneumonia. Two loops of the isolated culture injected subcutaneously into a rabbit were fatal in five days.

Case 2.—Rusty sputum from a case of pneumonia. One loop of the isolated culture injected intraperitoneally into a rabbit was fatal in three days.

Case 3.—Saliva from a healthy individual. One loop injected subcutaneously into a rabbit was fatal in five days.

Stuertz followed the virulence of the pneumococcus in the course of lobar pneumonia by injecting 1 c.c. of sputum subcutaneously into mice weighing 15 to 16 gms. The sputum was collected into sterile dishes, and 1 c.c. was drawn into a hypodermic syringe from different parts of the sputum. He followed

[1] Methods and protocols not published.

20 cases, in 11 of which he constructed charts showing the virulence. In the three cases with crisis, there was a rapid fall of virulence, but not until 24 hours after the drop of the temperature. He therefore concludes that the loss of virulence cannot be the cause of crisis. In the cases with lysis, the fall of virulence was more gradual and more or less parallel with the temperature curve. He also found that extensions of the disease are accompanied by increased virulence, and that we can detect this extension earlier by mice inoculation than by physical signs. Hence he thinks this has a prognostic value, since it puts the physician on his guard at critical moments.

Park and Williams placed a portion of sputum or material to be studied into serum broth at 36° C. for 24 hours. Of this they injected 3 to 4 c.c. subcutaneously into rabbits. Of the culture isolated from the blood at autopsy, and incubated 24 hours in serum broth, 0.1 and 4 c.c. respectively were injected into the ear veins of two rabbits. They also isolated cultures from the original material by means of blood agar plates, and after 24 hours at 36° C., colonies were transferred to blood agar slants and incubated. From these, transfers were made to serum broth, and after 24 hours' growth, tests for virulence were made as above. Isolations by the plate method were found to be distinctly less virulent than those by animal inoculation.

Their results as to the comparative virulence of the pneumococcus obtained from cases of pneumonia and from healthy individuals, with strains isolated by animal inoculation were as follows:

Amount Inoculated	Pneumonia Cases	Healthy Individuals
0.1 c.c.	51 per cent were fatal	31 per cent were fatal
4.0 c.c.	87 " " " "	69 " " " "

Eyre, Leathem, and Washbourn isolated strains of pneumococci by injecting lung juice or saliva subcutaneously or intra-peritoneally into rabbits and transferring the heart's blood at autopsy to blood agar tubes. The resultant growth was tested for virulence.

A) *One case of fatal lobar pneumonia:*

1 loop inoculated intraperitoneally was fatal in 3 days. Large number of cocci in blood.

.001 loop inoculated intraperitoneally was fatal in 4½ days. Fair number of cocci in blood.

.000,001 loop inoculated intraperitoneally was fatal in 4 days. Very few cocci in blood.

B) *Three cases from saliva:*

1) Saliva from case of carcinoma:

1 loop	inoculated	subcutaneously	killed	in	6	days,	heart's blood,	nil.
.001	"	"	"	"	9	"	"	"
.000,001	"	"	"	"	8	"	"	"

2) Normal saliva from a case of occupation paralysis:
With the above doses, the animals died in 11, 6, and 8 days respectively.

3) Normal saliva from a case of interstitial nephritis:
With the above doses, the animals died in 12, 33 days, and 24 hours respectively.
The last was accidentally injected intraperitoneally.

Longcope and Fox isolated three groups of organisms:

A) 16 cultures were obtained from pathological material, such as blood, consolidated lungs, empyema, otitis media, spinal fluid, and endocarditis. They all coagulated inulin and had capsules. In this group they do not state how the organisms were isolated.

B) 35 strains were obtained from healthy individuals by inoculating 2 c.c. of saliva subcutaneously into white mice. At autopsy, cultures were made from blood and from tissues.

a) 19 coagulated inulin and had capsules.

b) 16 did not coagulate inulin or show capsules.

In testing these strains for virulence no definite routine was followed. Some were inoculated subcutaneously, others intraperitoneally. The weight of the rabbits varied from 300 to 2,240 gms. The dose used for inoculation was not constant, but varied from 0.1 c.c. of a broth culture to three tubes of blood agar slants. No minimal lethal dose was determined. The following table was compiled from the results which they obtained in testing the strains of the three groups for their virulence in rabbits:

No. of Rabbits Inoculated	Dose Employed	A		B			
				a)		*b*)	
		Fatal	Not Fatal	Fatal	Not Fatal	Fatal	Not Fatal
1.......	0.1 c.c. 24-hr. broth culture	1	....	..	....	..	....
1.......	0.2 c.c. " " " "	1	....	..	....	..	....
3.......	0.5 c.c. " " " "	3	....	..	....	..	....
6.......	1. c.c. " " " "	4	....	1	....	..	1
9.......	2. c.c. " " " "	3	....	2	2	..	2
4.......	4. c.c. " " " "	1	....	2	1	..	....
16.......	1 tube 24-hr. growth blood agar	3	....	4	2	7	....
4.......	3 tubes " " " " "	..	....	1	....	1	2

Strains in group *a*) were, as a rule, much less virulent than those in group A, and exaltation of virulence was at times difficult.

Strains in group *b*) showed a very slight grade of virulence; in large doses they did sometimes kill rabbits, but the organisms could rarely be cultivated from the organs at autopsy.

Duval and Lewis isolated their organisms by means of the plate method. Agar plates were used to which were added drops of defibrinated blood by means of a sterile pipette. They tested the virulence of 35 recently isolated cultures obtained in large part from pneumonic lungs. Young rabbits were injected with either 10 c.c. of a 24-hour growth on glucose broth, or with the growth of a 24-hour culture on glucose agar plus rabbit's blood. The injections were usually made into the peritoneum. Only one fatal result followed. They found that when they injected the original material into animals their results fell into two classes. If the original material was in pure culture (as determined by plate method) the animal survived large quantities of material. If the original material was a mixture of bacteria the injection of a small dose usually gave a fatal result in one to four days. They also observed that faulty conclusions were drawn if strains were used which had been isolated by animal inoculations.

Buerger isolated 51 strains of pneumococci from the mouth by means of serum glucose agar plates. The material from which these isolations were made was obtained by means of sterilized swabs, which were rubbed against the pillars, tonsils, and posterior pharyngeal wall. The virulence of these strains was determined by injecting a 24-hour growth of one tube of serum agar slant, suspended in 1 c.c. normal saline solution. Injections were made subcutaneously into the backs of white mice. Strains were only two or three generations removed from the original plates.

His results were as follows:

	Virulent	Avirulent	Percentage	Percentage
38 strains isolated from normal throats	30	8	79	21
13 " " " throats of pneumonic cases	10	3	77	23

Jürgens used the same method as Stuertz. He selected cases uncomplicated by such factors as heart disease, nephritis, pregnancy, etc. His conclusions were that there must be other factors besides the virulence of the organism, since the organisms iso-

lated from those getting well often had a higher virulence than the strains isolated from cases proving fatal. He found also, that the virulence usually increases when there is progression of the disease, but claimed that at most it is found only several hours before the physical findings, whereas the pathological process must consume more than 24 hours before the physical findings become apparent. He states that we cannot draw conclusions as to the course of the disease by determining the virulence on mice.

Graffagnini injected 1 gm. of sputum and 1 gm. of normal salt solution subcutaneously into mice. The injections were made every day during the height of the disease, and every second or third day after the fall of temperature. Forty cases were studied. In all the maximum virulence occurred during the first four days, after which there was a tendency of the virulence curve to drop, except in fatal cases, in which mice died in seven to eight hours. Whether the temperature fell by crisis or by lysis, the virulence of the sputum always followed a uniform course, diminishing gradually, but never decreasing with a suddenness comparable to the critical fall of the temperature. This led him to conclude that crisis is not due to a diminution of the virulence of the pneumococci, but to the slowly accumulating powers of defense in the body. The following experiments show the results obtained:

Experiment 1.	8th	day	crisis,	mouse	died	in 12	hours.
	12th	"	"	"	"	24	"
	16th	"	"	survived.			
Experiment 2.	6th	day	crisis,	mouse	died	in 15	hours.
	8th	"	"	"	"	20	"
	9th	"	"	"	"	..	
	10th	"	"	"	"	24	"
	12th	"	"	"	"	36	"
	15th	"	"	survived.			

This review has shown the great diversity of results obtained by the different observers, which diversity is probably due to difference in methods employed. With two exceptions, the pneumococcus was isolated by inoculating animals. Many based their results simply on inoculation of sputum. Arbitrary doses were chosen by some, while others used variable doses. If a large enough dose is injected, all cultures may kill, and hence it is

necessary not only to establish an arbitrary dose, but to determine a minimal lethal dose.

Statistics of comparative virulence of strains isolated by animal inoculation and subsequently tested on the same species by inoculating a pure culture are misleading, for we know that animal inoculation enhances the virulence, but not necessarily to the same degree. It has been noted that in some strains a few passages will raise the pneumococcus to its maximum virulence, while in others it requires numerous passages, and in still others the virulence remains unchanged.

There are numerous objections to obtaining the virulence curve of the pneumococcus during the course of pneumonia by injecting into mice, at stated intervals, a definite weight of sputum. Mixed cultures are used. Toxic or aggressin-like substances are introduced. The number of bacteria vary in different specimens and different portions of sputum. This is especially true after crisis, when the pneumococci disappear from the sputum so rapidly that it is difficult to obtain a strain, whereas before crisis, the blood agar plates show almost a pure culture. This alone would account for the rapid fall of virulence after crisis as determined by this method. It is also difficult to follow the virulence after crisis because of the absence of sputum.

METHOD.

In this research the plate method has been employed in isolating the pneumococcus for the reasons already given. The argument usually brought forward against this method is that the pneumococcus rapidly loses its virulence on artificial media. To meet this objection the following authorities are cited.

Foa retained the virulence of the pneumococcus for 60 days in blood from a rabbit after inoculating the blood 24 hours and keeping it in a cool, dark place in a sealed glass tube.

Bunzl and Federn found that the pneumococcus grew luxuriantly in ascitic fluid and kept its virulence for three months.

E. Fraenkel and Reiche succeeded in keeping pneumococci virulent and viable for months by using agar, streaked with blood.

Spolverini retained the virulence of pneumococci in sputum from 55 to 140 days.

Eyre and Washbourn retained the original virulence of three cultures on blood agar 15, 25, and 63 days respectively.

Rosenow found that by using blood agar slants he could retain a moderate degree of virulence as long as 250 days. In two instances, a high grade of virulence was retained as long as 149 and 163 days respectively.

Carapelle and Gueli found that by growing the pneumococcus on blood serum, they could not only retain the virulence, but could gradually increase it.

My results agree with these findings. Three strains were kept on blood agar slants at 10° C. for 100, 109, and 111 days respectively without loss of the original virulence.

ISOLATION AND IDENTIFICATION.

The sputum was collected in sterile Petri dishes and plated immediately as follows: Selected portions of this sputum were washed in three to six successive solutions of sterile broth and macerated in a tube of broth. From this suspension two to four loops respectively were placed on two fresh blood agar plates and spread evenly over the surface by means of a sterile bent glass rod. To facilitate an even distribution of the bacteria, a few drops of broth were added to each plate. When expectoration had ceased, the culture was obtained by means of a bent swab which was introduced into the lower pharynx without touching any part of the mouth. This swab was then agitated in a tube of broth, and from this suspension, two plates were prepared. For identification, I relied on the characteristic green colonies on blood agar plates as described by Schottmüller and Rosenow, the ring-shaped colonies described by Buerger, the growth in milk, the fermentation and coagulation of inulin-serum water, the morphology, and the capsule stain.

DOSAGE.

The injection of a definite weight of sputum is open to the objections stated above. The usual method is to take a certain portion of a growth from solid media, such as a loopful; a fraction of a 24-hour growth on an agar slant such as 1/10, 1/5, 1/2, etc.; or

if liquid media is employed, a certain number of c.c. or fractions of a c.c. of a 24-hour growth. The objection to these two methods is that the pneumococcus is very susceptible to the slightest difference in reaction, or quality of the medium. It was noted many times in this investigation that even slight elevations of the temperature above 37° C. prevented growth. Kruse and Pansini, Eyre, Leathem, and Washbourn, and others found that the most virulent strains were those which were the most delicate and sensitive on artificial media, and that the less virulent ones were much less delicate, and could flourish under conditions in which the virulent strains were unable to grow. There is also a marked difference in the rate of growth of different strains.

The nephelometers of McFarland, and of Wells and Richards, were considered. One objection to nephelometers is that the error varies with the density of the solution and is adaptable only to low dilutions. On account of the small quantity of fluid to be injected, the emulsions used in this research had to be relatively heavy. Another objection is that it is difficult not to rub off some of the medium or to get away from the coloring of the blood agar, all of which vitiate the result.

There are two methods of direct enumeration, that of Winslow and Willcomb, and that of Wright. Winslow and Willcomb give a very good idea of the comparative value of counting the number of bacteria by the plate method, and by the direct enumeration on coverslip. The plate method was not available for this research for obvious reasons.

In this work Wright's method has been chosen because it is the most accurate known to the writer. One thousand red cells were counted, and a blood count was made each time from the same drop of blood from which the slide was prepared.

The pneumococcus is very readily emulsified, so much so, that if a strain was obtained which showed any tendency to flake or clump, grave doubts were entertained as to its identity. With carefully prepared slides the error is therefore slight. Below are the actual figures of the first four counts of two observers, each counting 500 cells.

Red Cells	Bacteria
500	89
500	83
500	99
500	85
500	45
500	55
500	49
500	52

As only 24-hour growths were used nearly all of the organisms were alive.

After determining the number of bacteria per c.mm., the emulsion was diluted with normal saline solution so that 1/20 c.c. represented 20,000,000 bacteria. This was done by drawing the necessary quantity of the emulsion into a 1 c.c. Sub. Q. tuberculin syringe, graduated into 100 parts as described below, and then rinsing out the syringe with the quantity of normal saline needed to bring the emulsion to the standard required.

Five mice were injected with 20, 40, 80, 160, and 320 million bacteria respectively. By this means, the variations in the individual susceptibility of the animals was controlled. The lowest dose selected was one which was not fatal, or fatal only after many days, usually as a result of toxemia. By considering the time of death, a composite curve of virulence could be constructed.

AMOUNT OF FLUID INJECTED.

The amount of fluid injected should be small. If one liter of normal saline is introduced subcutaneously into a man weighing 150 pounds, 1/57 of his body weight has been injected; while 1 c.c., into a mouse weighing 20 gms., is equal to 1/20 of its body weight. The quantity of fluid injected should certainly not exceed this. Large amounts of saline also have an aggressin-like action.

METHOD OF INOCULATING PRECISE AMOUNTS.

A 1 c.c. safety Sub. Q. tuberculin syringe graduated into 100 parts was used. Syringes were selected with a caliber of about four mm. This made the calibrated portion of a 1 c.c. syringe

about 80 mm. long. The syringe was divided into 20 main divisions, making each division, or 1/20 c.c. column, four mm. long, and hence not difficult to read.

Rosenau has shown that the loss in the mixing graduate is, on an average, about .0192 per cent. This loss would hold only if the dose for each mouse were in a separate container. The emulsion was made in a 5 c.c. test tube. After being standardized to 20,000,000 bacteria in 1/20 c.c., the syringe was loaded from this emulsion for all injections. Four mice could be injected from one loading, i.e., 1/20 c.c., 2/20 c.c., 4/20 c.c., and 8/20 c.c. respectively. The fifth mouse received 16/20 c.c., and reloading of the syringe from the same emulsion was necessary.

The loss in the syringe is from leaks at joints and at packing, from contact with glass and with surface of packing. By first drawing some air into the syringe, and then loading it with the emulsion, the leak at packing of piston, and also the loss by contact with packing was avoided. By using this *air cushion*, a meniscus was formed at junction of fluid and air, making the reading absolutely accurate. By simply twisting or rotating the piston, no difficulty was experienced in stopping at any one point. The usual method has been to use a rubber bulb in place of the piston, but a bulb is difficult to control, and by its use air is often introduced into the animal. The loss at needle end of syringe is obviated by having the thread molded into the glass, so that there are no connections to leak. The loss by adherence to sides of glass must be constant with each series, and would vary in the different series only if the glass were not clean.

MANNER OF CONSTRUCTING CHARTS.

The virulence curve is placed above the temperature curve, using the temperature line of 108 as the axis of abscissae. The points on the ordinates below, represent the number of hours in which the animal died from a given dose. Every degree of temperature represents 24 hours. Each point on the abscissae represents the hour of the day at which the sputum was collected.

HISTORIES AND PROTOCOLS.

In the first experiments, complete protocols are given showing the time of isolation, number of transfers made before inoculation, and the method of the standardization of the emulsion. All cultures fermented and coagulated inulin-serum water unless otherwise stated. Daily transfers were made on fresh blood agar tubes, and 24-hour growths were used for inoculation.

Case 1.—Acute lobar pneumonia. Left lower lobe. Child, aet. 11. History: Onset March 23 with otitis media, patient was deaf, and the drum head was inflamed and bulging. March 24, patient complained of a severe pain in left side and epigastric region. No physical signs were obtained by percussion or auscultation. March 25, sputum was prune juice in color, the leukocytes were 40,000, and physical signs became evident in the left lower lobe. March 26, about 8 P.M. patient had a typical crisis, and temperature dropped to normal in four hours, with profuse perspiration. March 27, there was a slight rise without any complications, and patient made an uneventful recovery.

EXPERIMENT 1.

March 25. 8 A.M. The sputum which was tenacious and prune juice in color, was collected into a sterile test tube and immediately plated on two blood agar plates.

March 26. Two typically green colonies were isolated from plates and transplanted to blood agar tubes.

March 27. From the blood agar tubes, transfers were made to blood agar, milk, and inulin-serum water.

March 28. Milk was typically acid, the inulin fermented, and the serum water coagulated. From the 24-hour growth of the blood agar tubes, a thick emulsion was made in 1 c.c. normal salt solution. By means of a capillary pipette, equal parts of emulsion, blood, and saline were thoroughly mixed on a slide, and from this smears were made on two slides. These were stained with Hasting's stain. There were 5,336,000 red cells per c.mm. in the drop of blood used. In an equal number of fields on slide, there were 1,046 red cells and 58 bacteria. Hence in 1 c.mm. of red cells there were 295,877 bacteria. In 50 c.mm. of red cells (one main division of tuberculin syringe, or 1/20 c.c.) there were 14,793,850 bacteria. To standardize the emulsion so that one main division of tuberculin syringe, or 1/20 c.c. is equal to 10,000,000 bacteria, take $\frac{10{,}000{,}000}{14{,}793{,}850}$ or 0.675 c.c. emulsion, or

67.5 marks of a tuberculin syringe graduated into 100 parts of emulsion.
32.5 " " " " " " " 100 " " salt solution.
1 main division of syringe, or 1/20 c.c. = 10,000,000 bacteria.
2 " " " " " 2/20 " = 20,000,000 "
4 " " " " " 4/20 " = 40,000,000 "
8 " " " " " 8/20 " = 80,000,000 "

March 28. Each of the above doses was injected into a mouse weighing 18 gms.

Mouse 1	receiving	10,000,000	bacteria	died	in	26	days.
" 2	"	20,000,000	"	"	"	22	"
" 3	"	40,000,000	"	"	"	48	hours.
" 4	"	80,000,000	"	"	"	28	"

Autopsy records:

Mouse 1 died from hemorrhage into the pleural cavity. No organisms were recovered.

Mice 3 and 4 showed numerous capsulated diplococci in smears and pure cultures were recovered from the heart's blood.

EXPERIMENT 2.

March 27. Twelve hours after crisis, the expectoration from lungs was collected into a sterile test tube, and plated immediately on blood agar.

March 28. Two typically green flat colonies were transferred to blood agar.

March 29. From the blood agar tubes, transfers were made to blood agar, inulin-serum water, and milk.

March 30. The inulin was fermented, the serum water coagulated, and the milk acid. Transfers were made from the blood agar to several blood agar tubes.

March 31. An emulsion was made and the slides prepared as in the preceding experiment.

The red cell count from the drop of blood from which the slide was prepared was 5,048,000. The count in an equal number of fields on slide showed 1,181 red cells and 381 bacteria.

Hence in 1 c.mm. of emulsion there were 1,628,524 bacteria.
" 1/20 c.c. " " " " 81,426,200 "

To standardize the emulsion, so that 1/20 c.c. = 20,000,000 bacteria, take $\frac{20,000,000}{81,426,200}$ of 2 c.c. or 0.4895 c.c. emulsion, or

48.95 marks of a tuberculin syringe graduated into 100 parts of emulsion.
151.05 " " " " " " " " " " salt solution.

1 main division of syringe, or	1/20 c.c.	= 20,000,000	bacteria.
2 " " " " "	2/20 "	= 40,000,000	"
4 " " " " "	4/20 "	= 80,000,000	"
8 " " " " "	8/20 "	= 160,000,000	"
16 " " " " "	16/20 "	= 320,000,000	"

March 31. Each of the above doses was injected into a mouse weighing 21 gms.

Mouse 1	receiving	20,000,000	bacteria	died	in	10	days.
" 2	"	40,000,000	"	"	"	27	hours.
" 3	"	80,000,000	"	"	"	27	"
" 4	"	160,000,000	"	"	"	48	"
" 5	"	320,000,000	"	"	"	48	"

Autopsy records:

Mouse 1 had a large, fresh, blood clot in each pleural cavity. Smears showed no organisms. Cultures on blood agar and milk remained sterile.

Mouse 2 showed numerous capsulated diplococci in blood from heart.

Mouse 3 had a large free blood clot in right pleural cavity. Smears from heart blood showed numerous capsulated organisms.

Mouse 5 showed numerous capsulated diplococci in blood from heart.

EXPERIMENT 3.

March 29. Two days after crisis the sputum was plated on blood agar.

April 1. An emulsion was prepared and standardized as in the two preceding experiments, and injected into five mice weighing 20 to 22 gms.

Mouse	1	receiving	20,000,000	bacteria	died	in	10 days.
"	2	"	40,000,000	"	"	"	28 hours.
"	3	"	80,000,000	"	"	"	$5\frac{1}{2}$ days.
"	4	"	160,000,000	"	"	"	6 hours.
"	5	"	320,000,000	"	"	"	6 "

Autopsy records:

Mouse 1. A large blood clot was found in each pleural cavity. No organisms were seen in smears, and the transfers to blood agar remained sterile.

Mouse 2. No organisms were found in smears made from the blood.

Mouse 3. Transfers of blood from heart to milk and blood agar gave pure cultures in both.

Mice 4 and 5. Numerous capsulated organisms were found in smears from blood.

EXPERIMENT 4.

March 31. Four days after crisis the sputum was plated on blood agar.

April 3. An emulsion was prepared and injected into five mice, each weighing 15 gms.

Mouse	1	receiving	20,000,000	bacteria	was alive 2 months later.
"	2	"	40,000,000	"	died in 8 days.
"	3	"	80,000,000	"	" " $6\frac{1}{2}$ "
"	4	"	160,000,000	"	was killed by accident.
"	5	"	320,000,000	"	died in 12 days.

Autopsy records:

Mouse 2. A blood clot was found in the pericardial sac and in each pleural cavity. Pure cultures were recovered from the heart's blood and from the blood clots.

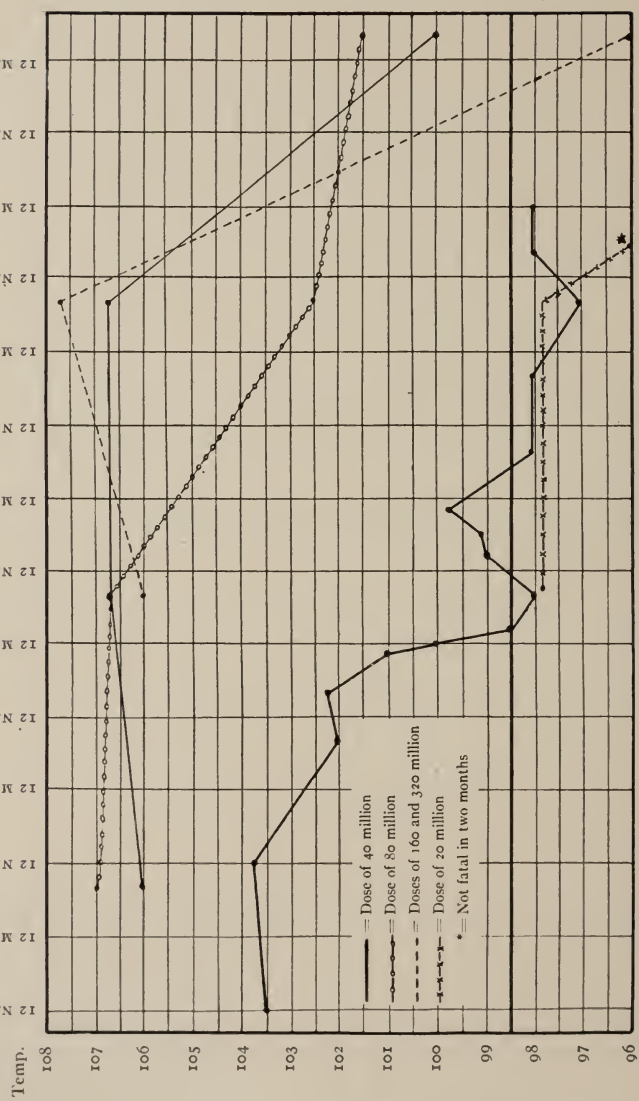
Mouse 5. A pure culture was recovered from the heart.

Chart 1 is a graphic representation of the temperature and virulence curves. While there was a typical crisis on March 26, the virulence of the organisms isolated on March 27 and 29 remained unchanged. The organisms isolated on March 31 were decidedly less virulent, as shown by experiment 4 and representation on chart.

Case 2.—J. S., aet. 44, carpenter. Acute lobar pneumonia, right lower lobe. Present illness began March 21 with a chill lasting two hours. The next day the patient had another chill, and developed a cough with pain in the right side. The sputum was streaked with blood. There were no complications, and the disease terminated by lysis with a slight elevation until April 11, due to delayed resolution.

CHART I.

CASE I.



* The dose of 20 million was continued until March 31; not fatal in 2 months.

EXPERIMENT 1.

March 28. The sputum was plated on blood agar.
March 31. Five mice were inoculated each weighing 20 gms.

Mouse	1	receiving	20,000,000	bacteria,	died in	6 days.
“	2	“	40,000,000	“	“ “	1 day.
“	3	“	80,000,000	“	“ “	2 days.
“	4	“	160,000,000	“	“ “	1 day.
“	5	“	320,000,000	“	“ “	2 days.

Autopsy records: Numerous capsulated diplococci were found in the smears, and pure cultures were recovered on blood agar.

EXPERIMENT 2.

April 6. The sputum was plated on blood agar.
April 9. The standardized emulsion was injected into five mice.

Mouse	1	weighing	19 gms.,	received	20,000,000	bacteria	and died in	15 days.
“	2	“	20 “	“	40,000,000	“	“ “ “	12 hours.
“	3	“	18 “	“	80,000,000	“	“ “ “	12 “
“	4	“	23 “	“	160,000,000	“	“ “ “	5 days.
“	5	“	23 “	“	320,000,000	“	“ “ “	12 hours.

Autopsy records:

Mouse 1. A large blood clot was found in the pleural cavity, from which a pure culture of diplococci was recovered on blood agar.

Mice 2, 3, and 5 had free blood clots in the pleural cavities, and also hemorrhage into the pericardium. No organisms were recovered on transfers to blood agar.

Mouse 4. Capsulated diplococci were found in the smears from the blood, and a pure culture was recovered on blood agar.

EXPERIMENT 3.

April 10. The sputum was plated on blood agar.
April 16. An emulsion was injected into five mice each weighing 15 gms.

Mouse	1	receiving	20,000,000	bacteria	died in	48 hours.
“	2	“	40,000,000	“	“ “	48 “
“	3	“	80,000,000	“	“ “	48 “
“	4	“	160,000,000	“	“ “	48 “
“	5	“	320,000,000	“	“ “	48 “

These mice were accidentally exposed to a cold wind and rain from an open window, which may account for the uniform time of death.

Autopsy records: Numerous capsulated diplococci were found in smears, and pure cultures were recovered on blood agar.

EXPERIMENT 4.

April 17. The sputum was plated on blood agar.
April 22. Five mice, each weighing 18 to 20 gms., were inoculated.

Mouse	1	receiving	20,000,000	bacteria	died in	10 days.
“	2	“	40,000,000	“	“ “	10 “
“	3	“	80,000,000	“	(not fatal)	
“	4	“	160,000,000	“	“ “	
“	5	“	320,000,000	“	“ “	

Autopsy records:

Mouse 1 had a large hemorrhage into the pleural cavity. The thorax and neck were placed in Zenker's fluid for a pathological study as to the origin of the hemorrhage.

Mouse 2 had a hemorrhage into the pleural cavity. No organisms were found in smears, and transfers from blood clot and heart remained sterile.

Chart 2 represents the temperature and virulence curves. The virulence remained unchanged until the temperature became normal. The probable explanation is that as long as physical signs are present, and pneumococci are expectorated from the lungs, the organisms remain virulent. When the physical signs disappear only such organisms as normally inhabit the mouth are obtained, and these become relatively avirulent because of some unfavorable environment. It is important to remember that the composition of saliva and that of sputum expectorated during the pneumonic process are entirely different; that while the pneumococcus may be kept alive for weeks in sputum, it rapidly dies in saliva.[1]

Case 3.—R. T., aet. 11. Acute lobar pneumonia, right upper lobe. Present illness began March 27 with a severe chill. Twelve hours later the patient became delirious, and on the following day, the sputum was blood tinged. The crisis occurred on the sixth day. There were no complications.

EXPERIMENT 1.

March 31. The sputum was plated on blood agar.

April 3. An emulsion was injected into five mice, each weighing 24 gms.

Mouse	1 receiving	20,000,000	bacteria	died in 2 days.
"	2 "	40,000,000	"	" " $2\frac{1}{2}$ "
"	3 "	80,000,000	"	" " 28 hours.
"	4 "	160,000,000	"	" " 3 days.
"	5 "	320,000,000	"	" " 36 hours.

Autopsy records: Numerous capsulated diplococci were found in smears from the blood.

EXPERIMENT 2.

April 2. 5:30 P.M. The blood-tinged sputum was plated on blood agar.

April 5. An emulsion was injected into five mice, each weighing 20 gms.

Mouse	1 receiving	20,000,000	bacteria	remained alive.
"	2 "	40,000,000	"	died in 24 hours.
"	3 "	80,000,000	"	" " 28 "
"	4 "	160,000,000	"	" " 24 "
"	5 "	320,000,000	"	" " 24 "

Autopsy records: Numerous capsulated diplococci were found in smears from the blood.

EXPERIMENT 3.

April 6. The sputum was plated on blood agar.

April 10. Five mice, each weighing 18 gms., were inoculated.

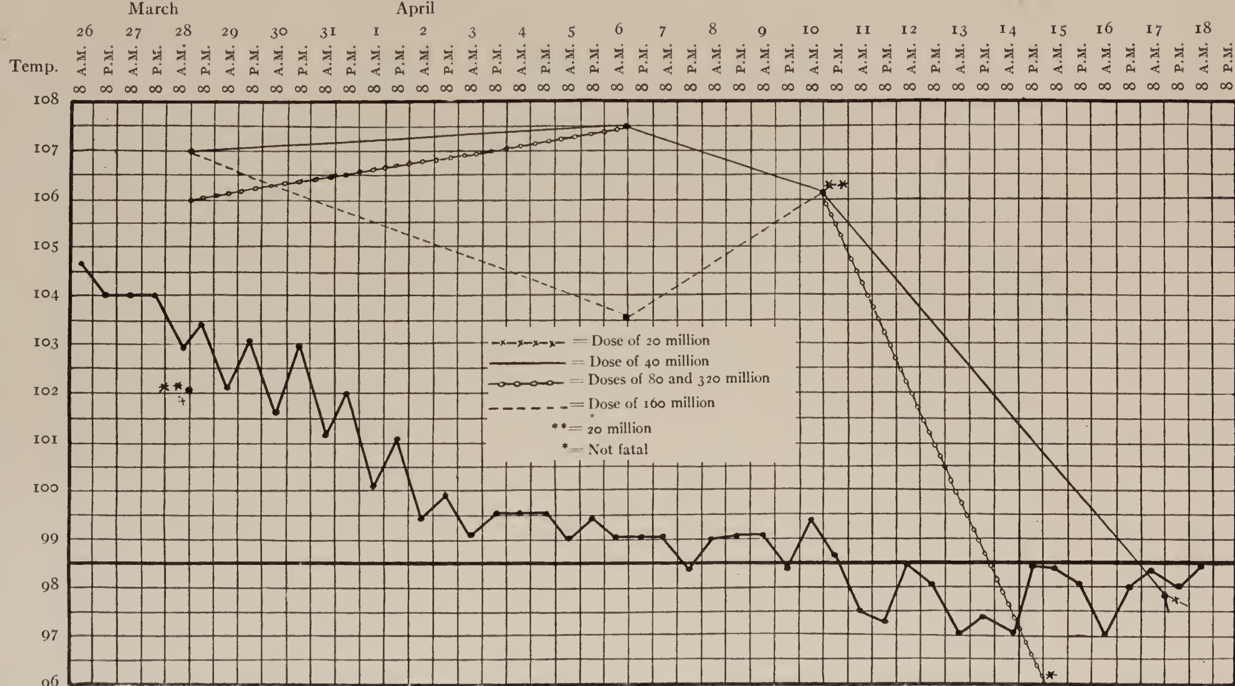
Mouse	1 receiving	20,000,000	bacteria	died in 24 hours.
"	2 "	40,000,000	"	" " 24 "
"	3 "	80,000,000	"	" " 26 "
"	4 "	160,000,000	"	" " 24 "
"	5 "	320,000,000	"	" " 24 "

Autopsy records: Numerous capsulated diplococci were seen in the smears from blood.

[1] For experiments in support of this view, see p. 318.

CHART 2.

Case 2.



** 20 million on April 6 ; hemorrhage in 15 days.

* A 20 million dose fatal in 10 days.

Experiment on April 17 in doses of 80 and 320 million; the chart not being extended so as to show this.

The dose of 160 million not fatal on April 17; the chart not being extended so as to show this.

EXPERIMENT 4.

April 12. As the patient had ceased to expectorate, a broth suspension was made from a pharyngeal swab and plated on blood agar.

April 15. An emulsion was injected into four mice, each weighing 18 gms.

Mouse 1	receiving	40,000,000	bacteria	died in 2 days.
" 2	"	80,000,000	"	" " 3 "
" 3	"	160,000,000	"	remained alive.
" 4	"	320,000,000	"	died in 4½ days.

Chart 3 represents the temperature and virulence curves as constructed from the data of the experiments. While there was a most typical crisis on April 2, without subsequent rise of temperature, or complication, the virulence of the organisms obtained from the sputum, nine hours after crisis, and four days after crisis, was the same as before crisis. With the disappearance of physical signs the patient ceased to expectorate, consequently the culture had to be made from a pharyngeal swab. This culture was relatively less virulent.

The notes made in the history in regard to the physical signs were as follows:

April 7. The consolidated area at the right apex is clearing. There are still numerous rales.

April 9. There is still a slight impairment of note, only a few rales are heard.

April 10. Only a few rales are heard. These disappear after several deep breaths.

April 20. The lungs are entirely clear.

Case 4.—J. Y., aet. 43. Acute lobar pneumonia (right upper and lower). Delayed resolution.

Past history: General health excellent.

Present illness began March 22 with pain in the right side, especially on taking deep breaths. At the same time he developed a cough, and began to expectorate a mucopurulent and slightly blood-tinged sputum. Two days later, he had two severe shaking chills. The leukocytes varied from 25,000 to 43,000. The temperature remained slightly elevated after crisis due to delayed resolution.

EXPERIMENT 1.

March 29. A fibrinous cast, expectorated into a sterile test tube, was plated immediately on blood agar plates, and on the following day the plate showed a pure culture of green pneumococci colonies.

April 3. Five mice, each weighing 15 gms., were inoculated.

Mouse 1	receiving	10,000,000	bacteria	died in 31 days.
" 2	"	20,000,000	"	remained alive.
" 3	"	40,000,000	"	died in 2 days.
" 4	"	80,000,000	"	" " 2 "
" 5	"	160,000,000	"	" " 36 "

Autopsy records:

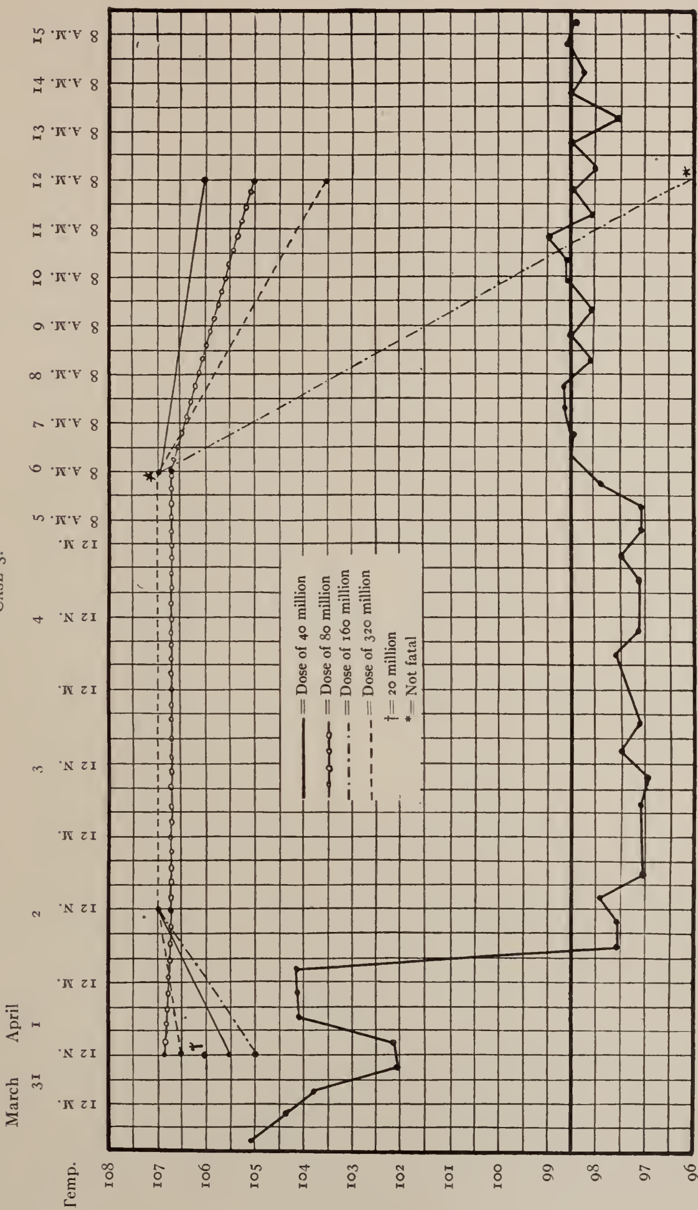
Mice 3 and 4 showed capsulated diplococci in smears, and pure cultures were recovered from the blood.

Mouse 1 had a large blood clot in the pleural cavity. No organisms were recovered from the blood.

Mouse 5 had a very large liver, studded with white areas. Smears showed numerous bacilli, possibly an invasion after death. The pneumococcus was not recovered.

CHART 3.

CASE 3.



† 20 million on April 2; not fatal.

* On April 6.

EXPERIMENT 2.

March 31. The sputum was plated at 6 P.M.

April 3. An emulsion was injected into five mice, each weighing 15 gms.

Mouse 1	receiving	10,000,000	bacteria	died in 5 days.
" 2	"	20,000,000	"	" " 3 "
" 3	"	40,000,000	"	" " 2 "
" 4	"	80,000,000	"	" " 18 hours.
" 5	"	160,000,000	"	" " 18 "

Autopsy records:

Mouse 1. A pure culture was recovered from the blood.

Mice 2 and 3. Capsulated diplococci were found in smears from the blood, and pure cultures were recovered.

Mice 4 and 5. Numerous capsulated diplococci were found in smears, and pure cultures were recovered from the blood.

EXPERIMENT 3.

April 4. The sputum was plated on blood agar.

April 8. An emulsion was injected into five mice, each weighing 15 gms.

Mouse 1	receiving	20,000,000	bacteria	remained alive.
" 2	"	40,000,000	"	died in 24 hours.
" 3	"	80,000,000	"	" " 24 "
" 4	"	160,000,000	"	" " 24 "
" 5	"	320,000,000	"	" " 24 "

Autopsy records: Numerous capsulated diplococci were found in the smears.

EXPERIMENT 4.

April 18. The sputum was plated on blood agar.

April 24. Five mice, each weighing 15 gms. were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in 2 days.
" 2	"	40,000,000	"	" " 2 "
" 3	"	80,000,000	"	" " 6 "
" 4	"	160,000,000	"	" " 3 "
" 5	"	320,000,000	"	remained alive.

Autopsy records:

Mice 1 and 2. Few organisms were seen in smears. A pure culture was recovered from the blood.

Mouse. 4. Numerous capsulated diplococci were seen in smears. A pure culture was recovered in the blood.

EXPERIMENT 5.

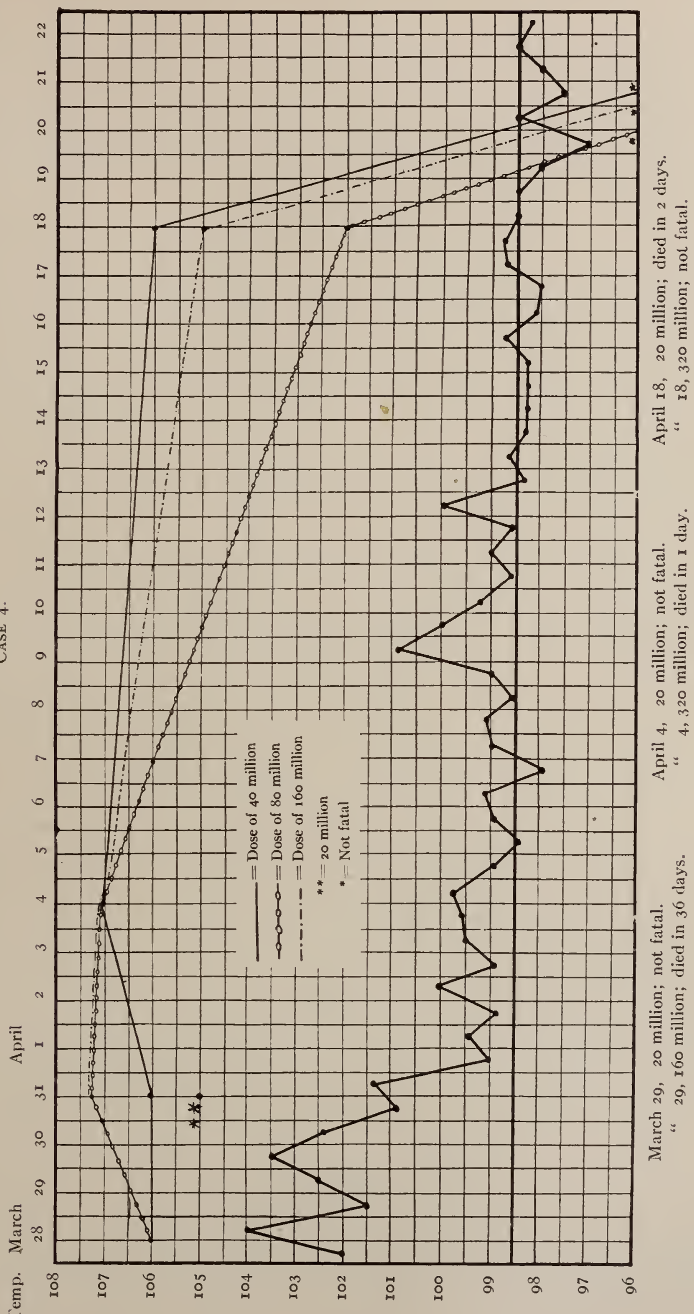
April 22. A broth suspension was made from a swab from the pharynx and plated immediately on blood agar. On the following day three green colonies were isolated. There was a typical growth in milk, but the inulin-serum water was not coagulated or fermented. The smears showed lanceolate diplococci which stained by Gram's method.

April 26. The growth on two blood agar slants was injected into a mouse without producing a fatal result.

Chart 4 shows the termination of the disease to be by lysis. The delayed resolution accounts for the rise of temperature until April 13. The virulence curve

CHART 4.

CASE 4.



shows that the organisms isolated during lysis were as virulent as those obtained during the active process. This virulence did not decrease until resolution was complete, and the temperature remained normal.

Case 5.—R. B., aet. 12. Acute lobar pneumonia (lower left). Present illness began April 15, four days before admission, with headache, fever, and pain all over body. On the fourth day patient was brought in an ambulance to the hospital. She had a typical crisis on the eighth day.

EXPERIMENT 1.

April 19. The blood-tinged sputum was plated on blood agar. On the following day, the plates showed an almost pure culture of green colonies. The growth was rather profuse and mucoid, and the plates appeared as though a small amount of water had been allowed to flow over the surface. The cultures isolated were of a variety of the pneumococcus usually called *Streptococcus mucosus capsulatus*. All of the colonies fermented and coagulated inulin, and rapidly acidified milk with late coagulation.

April 21. Five mice, each weighing 16 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in	2 days.
" 2	"	40,000,000	"	" "	2 "
" 3	"	80,000,000	"	" "	2 "
" 4	"	160,000,000	"	" "	1 day.
" 5	"	320,000,000	"	" "	6 hours.

Autopsy records: All of the mice showed numerous capsulated diplococci in smears.

EXPERIMENT 2.

April 21. 1 P.M. The sputum was plated. The resultant growth showed the same variety as that in experiment 1.

April 24. Five mice, each weighing 18 to 20 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in	36 hours.
" 2	"	40,000,000	"	" "	36 "
" 3	"	80,000,000	"	" "	24 "
" 4	"	160,000,000	"	" "	36 "
" 5	"	320,000,000	"	" "	36 "

Autopsy records: Smears from the blood showed large numbers of large capsulated diplococci.

EXPERIMENT 3.

April 22. The sputum was collected at 1 P.M. and plated on blood agar.

April 23. The plates showed a pure culture of green colonies of the variety *Streptococcus mucosus capsulatus*. Five mice, each weighing 15 gms., were inoculated.

Mouse 1	receiving	20,000,000	died in 36 hours.
" 2	"	40,000,000	" " 36 "
" 3	"	80,000,000	remained alive.
" 4	"	160,000,000	died in 36 hours.
" 5	"	320,000,000	" " 24 "

Autopsy records: All of the mice showed numerous capsulated diplococci in smears from the blood.

EXPERIMENT 4.

April 26. No expectoration was obtainable at the hour when it was wanted, so a broth suspension was made from a pharyngeal swab and plated on blood agar. On the following day, three colonies were isolated, all rapidly acidified milk, with partial coagulation in four days. The inulin was not fermented or coagulated. Smears showed typical lance shaped diplococci which stained by Gram's method.

April 30. Five mice, each weighing 15 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in 36 hours.
" 2	"	40,000,000	"	" " 36 "
" 3	"	80,000,000	"	" " 18 "
" 4	"	160,000,000	"	" " 18 "
" 5	"	320,000,000	"	" " 18 "

Autopsy records: Capsulated diplococci were seen in smears. Mouse 5 had a large hemorrhage into the pleural cavity.

EXPERIMENT 5.

May 1. A broth suspension was made from a pharyngeal swab, and plated on blood agar.

May 2. The plates showed a practically pure culture of green colonies. Three colonies were isolated, two of which fermented and coagulated inulin-serum water. The milk was rapidly acidified and partially coagulated within 24 hours. The smears showed lance shaped diplococci which stained by Gram's method.

May 4. Five mice, each weighing 15 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in 24 hours.
" 2	"	40,000,000	"	remained alive.
" 3	"	80,000,000	"	remained alive.
" 4	"	160,000,000	"	died in 24 hours.
" 5	"	320,000,000	"	" " 36 "

Autopsy records: Capsulated diplococci were found in smears from the blood.

Chart 5 shows that while a typical crisis occurred on April 20, the strains isolated one, two, and six days after crisis were as virulent as those isolated on the day before crisis. It also shows that there is no difference in virulence between the cultures obtained by pharyngeal swabs (experiment 4) and those obtained from the sputum expectorated (experiments 2 and 3). When the lung signs had cleared, the strain isolated from the pharynx was less virulent, two of the five mice inoculated surviving.

Case 6.—P. H., aet. 35. Acute lobar pneumonia (right upper, middle, and lower lobes). Present illness began March 31, with pain in back and right side. During the night a cough developed accompanied by a very severe headache. From that time the patient had fever, pain, and dyspnea. The sputum became blood tinged on April 5, two days after admission.

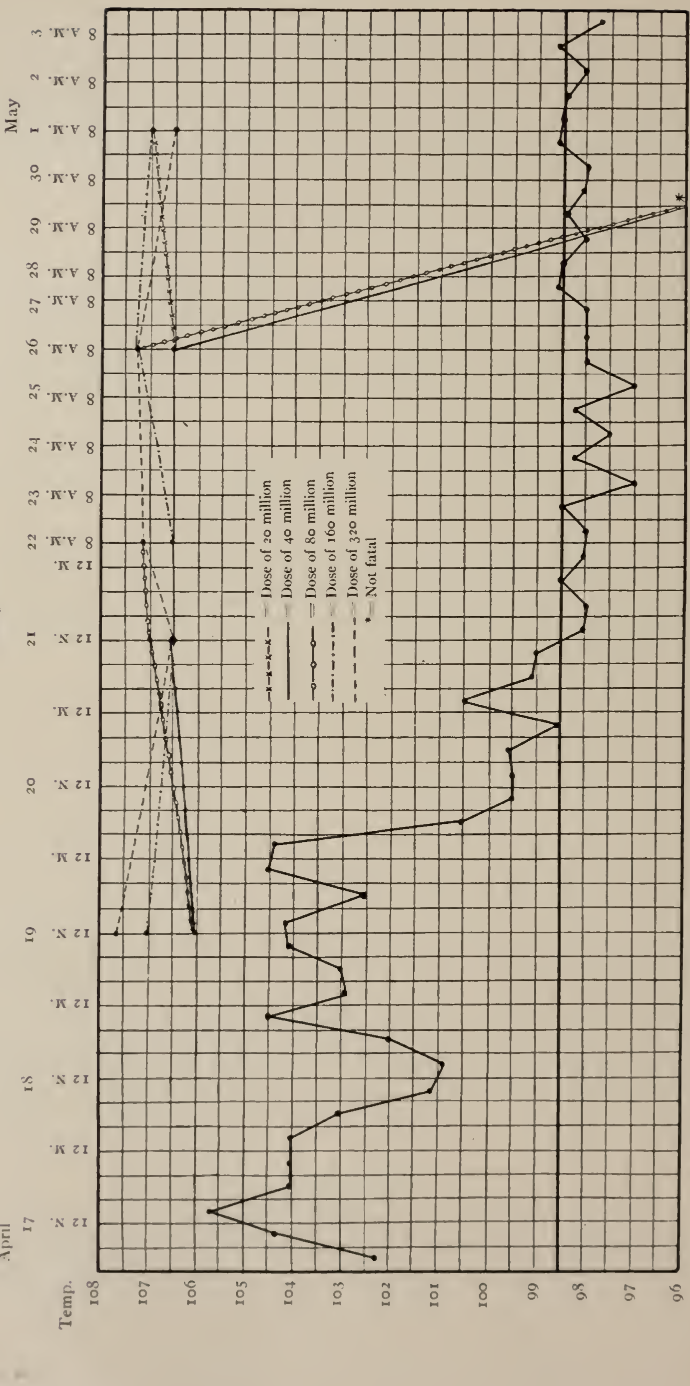
EXPERIMENT 1.

April 6. The blood-tinged sputum was plated on blood agar.

April 7. The plates were covered with colonies, majority of which were green. Several of these were isolated, and on the following day transferred to milk, inulin, and blood agar. The milk was rapidly acidified, but the inulin was only

CHART 5.

CASE 5.



April 22, 80 million; not fatal. Experiment made on May 1, not on April 29; the chart not being extended so as to show this.

partially fermented or coagulated. The organisms appeared as lance shaped pairs, but there was a marked tendency to form short chains. They stained by Gram's stain.

April 10. Five mice, each weighing 18 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in	2 days.
" 2	"	40,000,000	"	" "	2 "
" 3	"	80,000,000	"	" "	5 "
" 4	"	160,000,000	"	" "	3 "
" 5	"	320,000,000	"	" "	6 "

Autopsy records:

Mice 1, 2, and 3 showed numerous capsulated diplococci in smears from the blood.

Mouse 2 had a blood clot in the pleural cavity.

Mouse 4 did not show any organisms in smears from the blood.

Mouse 5 had a blood clot in the pleural cavity. No organisms were found in smears.

Experiment 2.

April 9, 5 P.M. The sputum was plated on blood agar. On the following day four green colonies were isolated. These rapidly acidified milk, but did not ferment or coagulate inulin-serum water. The organisms appeared as lance shaped diplococci with the same tendency to chain formation as in experiment 1. They stained by Gram's method.

April 13. Five mice, each weighing 18 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in	4 days.
" 2	"	40,000,000	"	" "	8 "
" 3	"	80,000,000	"	" "	3 "
" 4	"	160,000,000	"	" "	4 "
" 5	"	320,000,000	"	" "	6 "

Autopsy records:

Mice 1 and 4 were lost by accident after death.

Mice 2 and 5 had a large blood clot in the pleural cavity. No organisms were recovered by transfers to blood agar.

Mouse 3 did not show any organisms in smears from the blood.

Experiment 3.

April 11. The sputum was collected at 10 A.M. and plated on blood agar. On the following day, three typically green colonies were isolated, and transferred to milk, inulin, and blood agar. The milk was rapidly acidified, but the inulin-serum water was not coagulated or fermented. The organisms appeared as lance shaped diplococci, many of which formed short chains. They stained by Gram's method.

April 14. Five mice, each weighing 20 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in	6 days.
" 2	"	40,000,000	"	remained alive (May 18).	
" 3	"	80,000,000	"	died in	7 days.
" 4	"	160,000,000	"	" "	4 "
" 5	"	320,000,000	"	" "	4 "

Autopsy records:

Mouse 1 had a hemorrhage into the pleural sacs. No growths resulted from transfer of blood to blood agar.

Mouse 3. No organisms were recovered from the heart.

Mice 4 and 5 were lost by accident after death.

Chart 6 represents the temperature and virulence curves. Although a typical crisis occurred on April 9, the organisms isolated from the sputum during the crisis, and two days after the crisis, were as virulent as those before the crisis. No later cultures were taken in this case. The strains isolated from this case are unusual in that they did not ferment or coagulate inulin-serum water and showed a decided tendency to chain formation on blood agar. The chart also shows a very unusual low grade of virulence. With the exception of capsule formation in the animal (experiment 1) these cultures resemble the type of *Streptococcus viridans* described by Schottmüller.

Case 7.—G. S., aet. 17. Acute lobar pneumonia (right upper and lower lobes, left upper and lower lobes). Present illness began March 25, with profuse perspiration. The next day, a severe pain developed in the left side and abdomen. Three days after onset, the expectoration became blood tinged. The patient had no chills or convulsions. The leukocytes reached 72,000 on April 4. Crisis occurred on April 6.

EXPERIMENT 1.

April 1. The sputum was plated.

April 5. Five mice, each weighing 20 to 22 gms., were inoculated. (Mouse 5 weighed 27 gms.)

Mouse 1	receiving	10,000,000	bacteria	died in 48 hours.
" 2	"	20,000,000	"	remained alive.
" 3	"	40,000,000	"	died in 16 days.
" 4	"	80,000,000	"	" " 3 "
" 5	"	160,000,000	"	" " 9 "

Autopsy records:

Mouse 1. Myriads of capsulated diplococci were found in smears from the blood. A pure culture was recovered from the heart on blood agar.

Mouse 3 had been suffering from a paralysis for days. No hemorrhage was noted, and no organisms were recovered.

Mouse 4. Few organisms were seen in the smears. A pure culture was recovered from the blood.

EXPERIMENT 2.

April 4. The sputum was plated.

April 8. Four mice, each weighing 20 to 22 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in 36 hours.
" 2	"	40,000,000	"	" " 36 "
" 3	"	80,000,000	"	" " 36 "
" 4	"	160,000,000	"	" " 36 "

Autopsy records:

Mouse 1 had a large hemorrhage into the pericardium.

Mouse 2 had a large free blood clot in the pleural cavity.

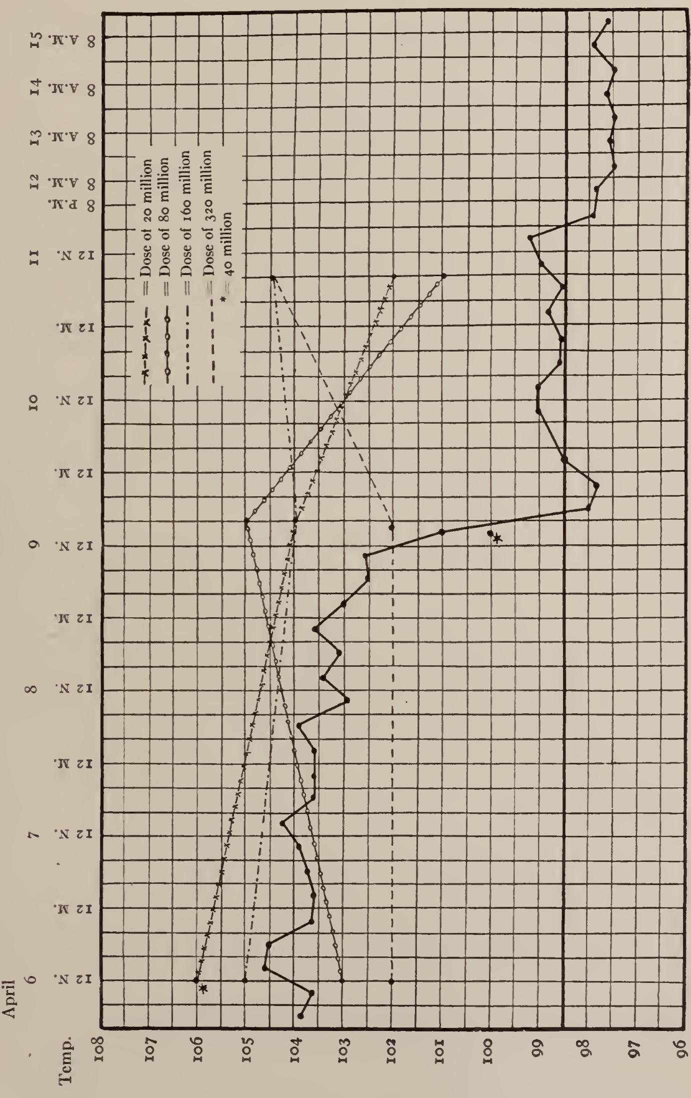
Pure cultures of capsulated diplococci were recovered from the blood in all of the mice.

EXPERIMENT 3.

April 7. The sputum was collected 24 hours after crisis, and plated on blood agar.
April 9. Five mice, each weighing 25 to 28 gms., were inoculated.

CHART 6.

CASE 6.



April 11, 40 million; not fatal.

Mouse 1 receiving	20,000,000	bacteria	died in	12 hours.
" 2 "	40,000,000	"	" "	12 "
" 3 "	80,000,000	"	" "	36 "
" 4 "	160,000,000	"	" "	$5\frac{1}{2}$ days.
" 5 "	320,000,000	"	" "	36 hours.

Autopsy records:

Mouse 1 had a hemorrhage into the pericardium.

Mice 1, 2, and 5. Capsulated diplococci were found in smears, and a pure culture was recovered on blood agar.

Mouse 3 had a large blood clot in the left pleural cavity, also a hemorrhage into the pericardium. Smears showed numerous capsulated diplococci.

Mouse 4 had a hemorrhage into the pericardium. A pure culture was recovered on blood agar. The sputum was plated on the 15th and 18th, but the cultures isolated were discarded because the strains were not typical.

Experiment 4.

April 19. The sputum was plated on blood agar, and on the following day, six green colonies were isolated.

April 21. Transfers were made to milk and inulin. Three of the strains gave a typical growth in milk, one strain constantly fermented inulin to a slight degree, but did not coagulate the serum.

April 24. A mouse was inoculated subcutaneously with the growth from two blood agar slants.

April 26. Mouse remained alive. The growth from two tubes was again injected.

May 4. The mouse died from a hemorrhage. A large blood clot was found in the pleural cavity. No organisms were recovered in smears or on transfers to blood agar.

(A young mouse, four weeks old, was inoculated with the growth obtained from two blood agar tubes. Death occurred in 18 hours, and myriads of definitely capsulated diplococci were seen in the smears.)

These experiments show that the virulence of the pneumococci isolated on the day after crisis was practically the same as before crisis. The virulence of the organisms isolated 13 days after crisis was of a very low grade.

Case 8.—L. C., aet. 29. Acute lobar pneumonia (left side.) Present illness began with a severe chill on April 18, followed by a sharp pain in the left side, and a marked shortness of breath. At this time he began to cough, expectorating a bloody sputum. The temperature varied from 102° to 105.° Lysis began on April 27, and the temperature was practically normal after May 2. There was a total suppression of the chlorides until April 29, with a marked rise after this date.

May 10. A broth suspension was made from a pharyngeal swab and plated on blood agar. On the following day six typical green colonies were isolated, all of which fermented and coagulated inulin-serum water, and acidified and coagulated milk. Smears showed a pure culture of lance shaped diplococci which stained by Gram's method.

April 15. Five mice, each weighing 15 to 17 gms., were inoculated.

Mouse 1 receiving	20,000,000	bacteria	remained alive.
" 2 "	40,000,000	"	" "
" 3 "	80,000,000	"	died in 3 days.
" 4 "	160,000,000	"	" " 1 day.
" 5 "	320,000,000	"	remained alive.

Autopsy records:

Mouse 4 showed a large free blood clot in left pleural cavity. Smears from blood showed few organisms, which were capsulated. Blood agar cultures from the blood clot and heart gave pure cultures of pneumococci.

Mouse 3 had a large free blood clot in the right pleural cavity, also in right pericardium. The origin of the hemorrhage seemed to be from the mediastinum. Smears from the clot and heart did not show any organisms. Transfers to blood agar failed to produce any growth.

These strains, isolated from the throat after the physical signs had disappeared, had a relatively low grade of virulence.

Case 9.—M. S., aet. 15. Acute lobar pneumonia (left side). Present illness began April 11 with a chill, followed by fever, nausea, vomiting, headache, and a sharp pain in the left side. Three days after onset he started to cough up a bloody sputum. Crisis occurred on the day of admission, April 17. There were slight elevations of temperature to 99.5° and 100° until May 9, after which his temperature remained normal.

May 11. A broth suspension was made from a pharyngeal swab, and plated on blood agar. On the following day seven colonies were transferred to blood agar. Only one of these strains fermented inulin and coagulated serum water. Smears from this culture showed typical lance shaped diplococci which stained by Gram's method.

May 15. An emulsion was made from a 24-hour growth on blood agar, and injected into five mice each weighing 16 gms.

Mouse	1	receiving	20,000,000	bacteria	remained alive.
"	2	"	40,000,000	"	" "
"	3	"	80,000,000	"	died in 24 hours.
"	4	"	160,000,000	"	" " 48 "
"	5	"	320,000,000	"	remained alive.

Autopsy records: Neither mouse showed any organisms in smears from heart, and no growth resulted from transfers to blood agar.

This experiment shows that a strain, isolated from the throat after the pneumonic process had cleared, had a relatively low grade of virulence.

On May 9, the hospital note on the condition of the lungs was as follows: "Lungs are clear throughout, except at left axilla, where the breath sounds are a little suppressed."

Case 10.—D. H., aet. 25. Acute arthritis of hip (infectious), chronic nephritis, hematuria. X-ray showed slight destruction of cartilage of joints. Temperature 99° to 101°.

May 10. A broth suspension was made from a pharyngeal swab and plated on blood agar. On the following day five typical green colonies were isolated, all of which fermented and coagulated inulin-serum water, and gave a typical growth in milk. Smears showed pure culture of lance shaped diplococci which stained by Gram's method.

May 14. Five mice were inoculated.

Mouse	1	(weight	17	gms.)	receiving	20,000,000	bacteria	remained alive.
"	2	"	17	"	"	40,000,000	"	" "
"	3	"	18	"	"	80,000,000	"	died in 3 days.
"	4	"	20	"	"	160,000,000	"	remained alive.
"	5	"	21	"	"	320,000,000	"	died in 3 days.

Autopsy records:

Mouse 5 had a large clot in the right pleural cavity.

Mouse 3 did not show any hemorrhage. The culture tubes to which transfers had been made were discarded by mistake.

This strain, obtained from a patient not ill with pneumonia, had the same relative virulence as the strains obtained from the throats of patients convalescing from pneumonia after the physical signs had disappeared.

Case 11.—D. P., aet. 20. Admitted March 10, 1910; died March 30. Lobar pneumonia, right side; bronchopneumonia left side, chronic fibrinous pleurisy, cloudy swelling of viscera; acute endocarditis, aortic valve; ulceration into right ventricle; acute splenic tumor; purulent meningitis.

March 20. The sputum was plated on blood agar. On the following day three green colonies were isolated, all of which fermented and coagulated inulin-serum water, and gave a typical growth in milk.

March 25. An emulsion was made and injected into four mice.

Mouse	1	(weight	21	gms.)	receiving	20,000,000	bacteria died in	4	days.
"	2	"	20	"	"	25,000,000	" " "	3	"
"	3	"	19	"	"	30,000,000	" " "	3	"
"	4	"	22	"	"	40,000,000	" " "	3	"

March 26. An emulsion was made from an organism isolated from the blood on March 20 and injected into four mice.

Mouse	1	(weight	18	gms.)	receiving	10,000,000	bacteria died in	5	days.
"	2	"	18	"	"	20,000,000	" " "	3	"
"	3	"	18	"	"	25,000,000	" " "	3	"
"	4	"	18	"	"	30,000,000	" " "	4	"

Conclusions: From this case it would seem that there is no difference in the virulence of the organisms isolated the same day from the sputum and from the blood.

ACTION OF SALIVA ON THE PNEUMOCOCCUS.

Experiment 1.

The organism tested was obtained from a blood culture from a case of pneumonia. As much of a 24-hour growth as adhered to a bent platinum needle was emulsified in (1) a tube of broth (10 c.c.). Of this suspension, six loops were transferred to (2) a second tube of broth.

The saliva was filtered through a Berkfeld filter which had previously been sterilized in the autoclave. This filtrate was collected in a sterile tube, and cultures from it remained sterile.

A. 1 c.c. of (2) was added to 1 c.c. of filtered saliva.

B. 1 c.c. of (2) was added to 1 c.c. of salt solution.

Plated six loops of each A and B, on fresh blood agar plates; (*a*) immediately; (*b*) four hours later; (*c*) eight hours after (*a*); and allowed them to incubate 24 hours at 37° C.

(*a*) Plated immediately	A=11 colonies
	B=20 "
(*b*) " after four hours	A=18 "
	B=too numerous to count
(*c*) " " eight "	A=8 colonies
	B=dry plate.

Experiment 2 (same technic).

(*a*) Plated immediately	A=38 colonies.
	B=35 "
(*b*) " after four hours	A=53 "
	B=80 "
(*c*) " " nine "	A=2 "
	B=426 "
(*d*) " " 21 "	A=0
	B=too numerous to be counted.

Sanarelli gives similar results for the staphylococcus with saliva filtered through a Chamberland filter.

From a staphylococcus culture as much as adhered to a needle was transferred to 10 c.c. of filtered saliva.

(*a*) Plated immediately	225 colonies.
(*b*) " after 12 hours	0 "
(*c*) " " 24 "	0 "
(*d*) " " two days	0 "
(*e*) " " three days	0 "

He states that saliva is a good culture media for the pneumococcus, that the organisms multiply rapidly, but also lose their virulence rapidly. He does not give any counts for the pneumococcus. Grawitz and Steffen confirmed this progressive and rapid loss of virulence in saliva, but found that this virulence is rapidly regained in pneumonic sputum. Their results were not controlled by plate counts. The loss of virulence may have been due to death of many of the organisms.

HEMORRHAGE IN MICE INOCULATED WITH THE PNEUMOCOCCUS.

No attempt was made to follow and describe the pathological processes. An autopsy was made in each case, smears were made from the heart's blood, also capsule stains. Transfers were made to blood agar slants and milk, for the recovery of the organism.

A condition which was very unusual was the presence of a fresh large blood clot, usually in both pleural cavities, also a large clot

in the pericardial sac. The blood seemed to come from vessels at the base of the heart or the hilum of the lung.

A specimen was submitted to Dr. Thomas P. Sprunt, instructor in pathology, whose report is as follows:

"Specimen submitted for examination is the body of a white mouse with massive blood clots in the thorax. The thoracic organs were fixed *en masse* in Zenker's fluid, embedded in celloidin and serial sections studied. Every tenth section was stained with hematoxylin and eosin, others were treated with Weigert's elastic tissue stain.

"There are large masses of blood in all the serous cavities, in the mediastinal tissues, and smaller hemorrhages in the lungs. The cause of the hemorrhage is quite apparent in the remarkable lesions found in the larger blood vessels. One of the most conspicuous of these is found at the point where a large artery is branching from the aorta. In one section, the whole sectional area of this large artery is affected, in other sections only one-half or one-third of its circumference. The lesion appears to be of irregular shape approximately 0.5 mm. in diameter. In some sections the edge of the pathological zone is quite sharp; in others the wall of the vessel diminishes in thickness gradually, finally losing entirely its normal structure of smooth muscle and elastic tissue and becomes continuous with a narrow delicate pink-staining tissue. It shows a faint nucleus here and there. With Weigert's stain this area is very striking. The elastic tissue of the aorta stains intensely and stops abruptly at the edge of the lesion. In the thin delicate tissue already noted there appear only a very few fragmented elastic fibres. The lumen of the vessel and the surrounding tissues are filled with blood and show no difference in this respect. These hemorrhages extend along the vessels in the mediastinal tissues and along the pulmonary vessels into the lung. Similar lesions are found in the pulmonary arteries in the interlobar spaces. The walls of these vessels are sharply broken apart and the hole thus formed in the wall is occasionally filled by a thrombus composed largely of platelets. Except in these sharply circumscribed areas the walls of the vessels seem perfectly normal. The lesions described are mainly confined to

the arteries which are composed of elastic tissue and smooth muscle. The veins in this animal show only cardiac muscle in their walls for a considerable distance from the heart and are therefore easily distinguished. In one of the smaller pulmonary veins there is a little pouch-like protrusion from its wall which is covered by tissue similar to that in the large artery already mentioned. Within the lungs, blood is collected in greatest amounts around the large vesssels, but there are also scattered hemorrhages in the lung alveoli. In association with these small hemorrhages, the alveolar walls are somtimes thickened and show many polymorphonuclear leukocytes. There are very few leukocytes within the alveoli. There is no apparent inflammatory process in the walls of the vessels. The lesion in the aorta and its large branch apparently consists of an atrophy of the tissues composing its walls. This atrophy is not so manifest in the smaller pulmonary arteries in which the rupture might possibly be explained by some sudden trauma." (Further studies on this condition are in progress.)

Out of 84 fatal cases, 24 show this large blood clot in one or all of the regions described above. The following tabulation gives the duration of life of the animals dying from hemorrhage after inoculation.

5 mice died in 1 day.	1 mouse died in 11 days.
4 " " " 2 days.	1 " " " 15 "
1 mouse died in 6 "	1 " " " 17 "
2 mice died in 8 "	1 " " " 19 "
2 " " " 10 "	5 mice " after 20 days.

The shortest period was one day, the longest 36 days. No organisms were recovered from the blood in the late cases. One case gave a positive culture as late as the sixth day, another as late as the eighth day.

EFFECT OF DOSE ON THE FREQUENCY OF HEMORRHAGE.

In seven, hemorrhage followed the injection of 20 million bacteria; in seven, the injection of 40 million; in four, the injection of 80 million; in one, the injection of 160 million; in three, the injection of 320 million. The reason for the hemorrhages occurring more frequently with the smaller doses is that the duration of life was longer as shown by the following:

Dose	Days before fatal hemorrhage occurred
20 million	2, 10, 10, 11, 15, 31, 36 days.
40 "	1, 1, 2, 8, 32 days.
80 "	1, 2, 2, 8 days.
160 "	6 days.
320 "	1, 2, 19 days.

CONCLUSIONS.

The following conclusions may be drawn from the experimental data of this investigation:

1. Crisis is not a result of any change in the virulence of the pneumococcus.

2. As long as pneumococci are expectorated from the lungs (for a short time in cases with crisis, for a longer period in cases with lysis) the organisms retain practically their original virulence.

3. Strains obtained from pharyngeal swabs during the pneumonic process have the same virulence as those obtained from the sputum.

4. Strains obtained from pharyngeal swabs after the lungs have resolved and expectoration has ceased, have a relatively low grade of virulence.

5. A strain obtained from the throat of a patient not ill with pneumonia, had the same relative virulence as strains obtained from the throats of convalescent patients in whom the physical signs had disappeared.

6. Loss of virulence of organisms obtained from the throats of patients who have recovered from pneumonia, seems to be due to an unfavorable action of the saliva.

7. From the results obtained in one case, there does not seem to be any difference in virulence of the organisms obtained from the blood, and those obtained from the sputum.

The writer wishes to thank Dr. L. F. Barker for the use of the cases from the medical clinic of The Johns Hopkins Hospital, and to acknowledge the suggestions of Dr. R. I. Cole, Dr. W. W. Ford, and Dr. P. W. Cough in the preparation of this report.

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SOME OBSERVATIONS ON THE BLOOD OF DAIRY COWS IN TICK-INFESTED REGIONS.*

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The material contained in this paper has been selected from a mass of data collected during the past two years. The subject was suggested by some observations made while conducting one of our Adams Fund projects. Our first set of results, obtained from a limited number of animals (15), when compared with the results of other observers in the hematological studies of the blood of normal cattle, brought to light some differences which could not then be explained. In view of this fact we thought it necessary to make a more thorough study in an attempt to account for these differences. In all over 50 individual animals have been examined, a few of which were subjected to repeated examination at various times of the year. Our observations during this period of time have not brought any change in the general result. We do not consider, however, that we have established a new normal, but according to data recently obtained we believe that what differences do exist between our data and those of other observers are entirely due to the influence of the Texas fever parasite upon the blood of the animals which have recovered from the disease. It is generally considered that the blood of cows that have had tick fever never attains the optimum condition that it maintained preceding an attack. To our knowledge nothing has been published in regard to the condition of the blood after recovery from Texas fever nor has anything appeared in the literature concerning the history of the leukocytes during or after an attack. It has, therefore, seemed advisable to publish our results.

TECHNIC.

The blood was procured from a gash made by a spring fleam. This gash was made on the rump at a place most accessible to the operator and at the same time in a region where the blood could be made to ooze out with readiness. When the hair is clipped

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and the site thoroughly cleaned a good specimen of blood can be obtained. With proper care a clean sample of blood can be taken from between the lips of the gash. The counts are materially affected when dirt particles, oil droplets, and debris are taken up in the pipette. Every instrument and piece of apparatus was placed in readiness so that the work could be done quickly. After the operation the wound was treated with five per cent carbolic acid and the lips held together for a few seconds. This precaution was finally given up as unnecessary since no septic conditions were produced and the scars soon disappeared.

The samples of blood were taken during milking time, between four and five o'clock in the afternoon, while the cows were in the stalls, feeding. A few animals were nervous, but the majority took little or no notice of the slight operation. The ages of the animals varied from one to 12 years. After the samples had been gathered they were taken to the laboratory and counts made. During the winter it was too dark to do this work at once, so they were held over until the following morning. There appears to be little or no difference between the morning and evening results, or, in other words, between results obtained one hour or 14 hours after the samples are taken. What differences do show up are within the limit of error. The accompanying table is inserted to show this fact.

Two samples from each animal in the dilution of 1:200 were taken. One was examined on return to the laboratory while the other was put away for examination in the morning. (P.M. = samples taken in the afternoon and counts made within one hour after samples were collected. A.M. = samples taken in the afternoon but held until the morning following and then examined.)

TABLE 1.

Number	P.M.		A.M.	
	Reds	Whites	Reds	Whites
1	5,880,000	12,000	4,584,000	13,554
2	5,120,000	15,554	5,240,000	8,888
3	4,142,000	7,332	4,936,000	11,776
4	4,608,000	6,000	5,440,000	7,332
Average	4,940,000	10,221	5,050,000	10,387

The above method would afford a slight chance for error. The samples were taken by two individuals. Those to be examined immediately, by one, and those for morning study, by the other. We next took samples in the afternoon and held them over until the morning following, when they were counted together with samples taken in the morning. The evening and morning samples were taken from the same individual animals. (P.M. = samples taken in the afternoon and counted in the morning. A.M. = samples taken in the morning and counted within an hour after taking. In this case the samples were all taken by one individual.)

TABLE 2.

Number	P.M.		A.M.	
	Reds	Whites	Reds	Whites
382	5,744,000	11,110	7,504,000	8,888
383	5,080,000	13,776	5,544,000	16,888
384	7,152,000	9,822	6,352,000	12,000
385	6,680,000	12,000	6,752,000	10,222
393	5,792,000	8,000	6,336,000	10,666
397	5,624,000	14,776	6,344,000	20,222
399	broken	broken	6,600,000	12,444
400	6,488,000	5,332	6,664,000	14,776
Average	6,080,000	10,688	6,512,000	13,263

The morning and evening counts are known to vary slightly.

Finally we took samples in the afternoon and within an hour from the time they were taken we began counting. After counting, the pipettes, which were about half full, were put aside and in the morning were counted again with the following results.

In this case all the samples were taken by one individual, and the morning and evening counts from the same pipette.

TABLE 3.

From Same Pipette.

Number	P.M.		A.M.	
	Reds	Whites	Reds	Whites
1	4,152,000	7,332	4,464,000	7,110
2	6,488,000	8,332	6,160,000	8,000
3	4,848,000	11,110	4,800,000	12,888
4	4,912,000	9,110	5,360,000	10,332
5	4,768,000	10,000	4,800,000	7,554
6	6,488,000	8,332	4,976,000	6,888
7	4,936,000	12,000	4,768,000	10,666
Average	5,227,000	9,460	5,047,000	9,062

We consider the above differences as within the limit of error.

The number of erythrocytes and leukocytes per c.c. was determined. Both sets of corpuscles were counted from the same preparation. The blood was diluted 1:200 with Toisson's fluid.

In this work the reds and whites were counted in the same preparation. To facilitate the work the dilution of 1:200 was used instead of the 1:100 dilution, as the number of red corpuscles to a square was large and rendered the counts tedious when so many examinations were made. We have found that the differences are small and fall within the limit of error. This may be observed in Table 4.

TABLE 4.

Name	Dilution 1:100	Dilution 1:200
	No. of Leukocytes per c.mm.	No. of Leukocytes per c.mm.
Estelle	11,777	......
Sweet Eyes	11,555	13,776
224	10,055	9,554
Maimie	12,500	10,666
228	12,111	13,844
Roxie	11,611	12,444
Marjorie R.	15,518	17,110
Lucy R.	7,296	8,444
Lady Doth	9,950	12,222
316	14,444	bad
Cecile S.	10,200	8,666
125	16,444	16,000
Wayne P.	8,000	8,222
116	10,332	9,222
100	15,777	16,666
Lura K.	15,888	15,332
Timola's Lassie	7,777	8,444
Average	11,837	12,040

The Thomas Zeiss hematocytometer with the Zappert Ewing ruling was used for counting both reds and whites. In estimating the number of red cells, one hundred squares were counted. This operation was repeated with another drop, and if the results varied more than 25 from that obtained with the first drop, a third preparation was made and the three results averaged. In counting the white cells the number in the whole ruled area or nine squares was counted. Two preparations were examined as described above in the counting of the red cells. Whenever necessary a third preparation was made and an average taken of all three. A differential count of the leukocytes was also made. Wright's modification of Jenner's stain was used exclusively.

The hemoglobin was determined by use of Dare's hemoglobinometer. In some cases the results were checked by another person. The results were in fairly close agreement. Results obtained in different months and different years tallied quite closely.

The following tables have been taken from Dr. Burnett's book on *Clinical Examination of the Blood of Animals.*

Red Corpuscles per c.mm.	Leukocytes per c.mm.	Hemoglobin per c.mm.	Specific Gravity	Size of Red Corpuscles	Authors
6,275,000	......	....	....	4.6–7.2 m	Bethe
6,152,000	5,486	59.7	....	....	Dimock and Thompson
....	......	....	....	5.95 m	Gulliver
4,200,000	......	....	....	6 m	Malassez
6,000,000	9,730	....	....	5–6 m	Smith and Kilbourne
5,073,000	......	....	....	....	Stoltzing
6,503,000*	7,841	....	....	....	Storch
6,683,000†	9,367	....	....	....	Storch
5,473,000‡	8,241	....	....	....	Storch
7,055,000‡‡	11,614	....	....	....	Storch
8,523,000§	15,739	....	....	....	Storch

* Bulls. † Oxen. ‡ Cows. ‡‡ Young cattle. § Calves.

Dimock and Thompson obtained the following numbers and percentages of the several varieties of leukocytes in the blood of normal cattle:

	Per c.mm.	Average per cent	Minimum per cent	Maximum per cent
Lymphocytes	2,992	54.2	31	76
Large mononuclears	86	1.4	0.2	3.3
Polymorphs	1,786	30.5	13.0	45.8
Eosins	772	13.15	3.8	26.5
Mast cells	31	0.59	0.1	1.2

Refik-Bey gives the normal number of leukocytes for cattle as 7,000–11,000 per c.mm., the number of mononuclears, including lymphocytes, as 4,500–6,500 per c.mm. (57–84 per cent), the number of polynuclears as 1,500–3,500 per c.mm.

The above is a summary of our present knowledge of the clinical work on the blood of cattle and is inserted here for purposes of comparison.

In our original work 15 animals were selected and placed in a feeding experiment. A clinical study of the blood was begun on October 22, 1908, and at first consisted in a count of erythrocytes only. The animals were heifers with their first calves. There were three lots of three each, making nine in all. Two months

TABLE 5.

All animals are females and grade Jerseys. The number of red corpuscles is given as million per cubic centimeter. The number of white corpuscles is given as thousand per cubic centimeter.

Number	Age in Years	Time Taken	Reds	Whites	Average Reds	Average Whites
382	$3\frac{6}{12}$	10–28–08	7.516	14.0	.....	.....
		11– 7–08	7.296	11.4	.....	.....
		11– 9–08	7.360	10.2	.....	.....
		11–16–08	7.536	13.2	.....	.....
		2– 8–09	7.696	10.2	7.480	11.8
383	$3\frac{10}{12}$	11– 4–08	7.680	24.0	.....	.....
		11–18–08	5.280	18.8	.....	.....
		12– 8–08	6.752	30.4	.....	.....
		1–12–09	6.640	28.8	.....	.....
		2– 9–09	5.400	14.3	6.350	23.3
384	$3\frac{7}{12}$	10–29–08	7.697	14.0	.....	.....
		11–10–08	6.800	7.4	.....	.....
		11–17–08	7.296	9.4	.....	.....
		11–23–08	7.600	8.0	.....	.....
		1–13–08	7.160	14.0	7.310	10.6
385	$3\frac{7}{12}$	10–29–08	5.960	.....	.....	.....
		11–10–08	5.936	9.4	.....	.....
		11–17–08	7.040	11.8	.....	.....
		11–23–08	6.560	8.4	.....	.....
		12– 9–08	6.288	8.4	.....	.....
		1–13–09	6.920	13.0	.....	.....
		2–10–09	7.160	9.3	6.552	10.0
399	$2\frac{10}{12}$	11– 2–08	6.320	20.00	.....	.....
		11–11–08	7.136	12.80	.....	.....
		11–18–08	7.000	11.00	.....	.....
		1–12–09	6.720	13.54	.....	.....
		2– 9–09	7.256	12.88	6.887	14.04

Number	Age in Years	Time Taken	Reds	Whites	Average Reds	Average Whites
393	$3\frac{6}{12}$	11– 2–08	5.520	14.22	.....	.....
		11–11–08	5.656	14.44	.....	.....
		11–18–08	7.160	14.00	.....	.....
		12– 8–08	5.752	12.44	.....	.....
		1–12–09	4.560	15.20	.....	.....
		2– 9–09	6.248	13.11	5.816	13.9
397	3	10–22–08	7.472	.....	.....	.....
		11– 7–08	3.397	10.0	.....	.....
		11– 9–08	5.384	14.0	.....	.....
		11–16–08	6.312	11.0	.....	.....
		12– 7–08	4.688	4.66	.....	.....
		1–14–09	6.480	15.4	.....	.....
		2– 8–09	6.080	11.7	.....	.....
		3– 9–09	5.480	17.5	.....	.....
		4– 6–09	6.744	14.0	5.782	12.29
398	3	10–29–08	7.440	16.22	.....	.....
		11–10–08	5.888	11.60	.....	.....
		11–17–08	7.256	12.00	.....	.....
		11–23–08	7.280	15.20	.....	.....
		2–10–09	7.216	11.76	.....	.....
		3–10–09	6.944	4.66	.....	.....
		4– 8–09	7.904	19.55	7.132	13.00
400	$2\frac{11}{12}$	10–22–08	7.256	12.88	.....	.....
		11– 7–08	6.312	20.80	.....	.....
		11– 9–08	6.984	12.00	.....	.....
		11–16–08	7.200	8.88	.....	.....
		12– 7–08	7.376	16.20	.....	.....
		1–14–09	7.104	14.80	.....	.....
		2– 8–09	6.944	15.77	7.025	14.47

General Average.

Number	382	383	384	385	393	397	398	399	400	Average
Reds	7.480	6.350	7.310	6.552	5.816	5.782	7.132	6.887	7.025	6.704
Whites	11.80	23.26	10.56	10.06	13.90	12.29	13.00	14.04	14.47	13.71

TABLE 6.

Number	Date	Reds	Whites	Percentage of Hemoglobin	Polynuclears		Mononuclears		Lymphocytes		Mast		Eosinophiles	
					Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
382	4– 6–09	7.152	8.88	82	329	3.7	...	...	8,178	92.1	80	0.9	275	3.1
	8– 5–09	7.280	8.44	76	2,583	30.0	287	3.4	6,060	71.8	101	1.2	675	8.0
	3– 8–10	6.552	12.44	100	1,593	12.8	722	5.8	9,034	72.6	75	0.6	1,020	8.2
	5– 4–10	7.2[illegible]4	12.00	94	1,044	8.7	156	1.3	9,636	80.3	48	0.4	1,032	8.6
	6–24–10	7.008	15.55	93	2,255	14.5	684	4.4	10,669	68.6	75	0.5	1,773	11.4
	11–14–10	6.320	11.44	95	2,060	18.0	...	...	8,537	74.6	23	0.2	824	7.4
	3– 4–11	6.736	11.27	91	1,173	10.4	180	1.6	8,615	76.4	...	...	1,285	11.4
383	3– 9–09	4.576	24.55	90	....	....	...	...	......	....	...	...	.....	....
	4– 7–09	5.280	22.66	74	1,243	4.6	101	0.4	20,852	92.0	...	...	680	3.0
	8– 5–09	3.456	17.77	85	1,635	9.2	604	3.4	14,114	79.4	...	...	1,067	6.0
	3– 9–10	4.392	21.11	..	1,393	6.6	211	7.0	18,155	86.0	213	1.2	253	12.0
	3–27–10	4.808	18.88	81	1,511	8.0	661	3.5	14,423	77.0	42	0.2	1,889	10.0
	5– 4–10	4.792	22.33	95	715	3.2	625	2.8	19,775	89.0	57	0.3	938	4.2
	6–24–10	5.480	22.88	80	2,724	11.9	572	2.5	17,182	75.2	179	0.8	2,083	9.0
	11–14–10	4.700	15.33	80	1,411	9.2	889	5.8	12,082	78.8	206	0.9	843	5.5
	3– 4–11	5.000	20.22	81	1,011	5.0	283	1.4	17,957	88.8	77	0.5	809	4.0
											162	0.8		
384	3–10–09	7.288	14.00	85	3,654	26.1	546	3.9	8,624	61.6	...	...	1,148	8.2
	4– 8–09	6.752	9.33	75	1,281	14.8	...	...	7,540	80.8	...	...	448	4.8
	8– 8–09	5.784	6.55	78	1,311	20.0	210	3.2	4,483	68.4	26	0.4	472	7.2
	3–10–10	5.520	9.76	80	1,152	11.8	254	2.6	7,422	76.0	39	0.4	869	8.9
	5– 6–10	5.184	8.00	..	1,968	24.6	96	1.2	5,120	64.0	112	1.4	672	8.4
	6–24–10	5.584	11.54	85	1,455	12.6	185	1.6	8,877	76.90	115	1.0	866	7.5
	3– 4–11	6.112	9.11	83	2,496	27.4	264	2.9	5,439	59.7	82	0.9	829	9.1
385	3–10–09	5.408	10.66	77	1,422	13.4	491	4.6	7,999	75.0	...	...	747	7.0
	4– 8–09	5.816	10.22	72	675	6.6	...	...	7,932	77.6	31	0.3	1,533	15.0
	8– 4–09	6.000	12.11	80	.....	....	...	...	......	....	...	...	.....	....
	3–10–10	5.856	11.55	89	2,623	22.7	716	6.2	7,152	61.9	173	1.5	1,040	9.0
	5– 6–10	6.224	10.11	91	1,152	11.4	131	1.3	7,582	75.0	71	0.7	1,152	11.4
	6–24–10	5.632	16.66	95	1,067	10.0	149	1.4	8,063	75.6	32	0.3	1,337	12.5
	11–14–10	5.680	11.44	88	2,380	20.8	343	3.0	7,507	65.6	69	0.6	1,213	10.6
	3– 4–11	5.048	12.66	88	836	6.6	215	1.7	10,842	85.6	38	0.3	785	6.2

393	3–10–09	6.176	14.22	87	113	8.0	455	3.2	11,605	81.6	...	...	1,024	7.2
	4– 7–09	4.336	10.66	83	.....	....	...	...	......	....	...	...	.....	...
	8– 4–09	5.760	15.55	90	1,493	9.6	321	2.0	12,505	80.4	120	0.8	964	6.2
	3– 9–10	4.528	14.22	85	1,010	7.1	...	1.58	13,088	81.8	96	0.6	1,184	7.4
	6–24–10	5.376	16.00	84	1,376	9.6	250	2.5	11,354	81.1	196	1.4	728	5.2
	11–14–10	5.816	16.00	84	1,408	8.8	80	0.5	14,112	88.2	...	...	368	2.3
	3– 4–11	5.784	8.00	86	296	3.7	80	1.0	7,280	91.0	...	...	320	4.0
397	8– 4–09	3.792	14.22	73	1,749	12.3	497	3.5	10,766	75.7	71	0.5	1,095	7.7
	3– 9–10	4.960	20.22	90	2,184	10.8	749	3.7	16,393	80.8	202	1.0	708	3.8
	5– 4–10	4.816	20.00	87	1,160	5.8	348	0.6	17,280	86.4	...	...	1,440	7.2
	6–24–10	5.740	27.66	90	2,984	10.8	442	1.6	23,820	86.2	...	...	387	1.4
	3– 4–11	6.496	17.77	88	1,955	11.0	356	2.0	14,252	80.8	71	0.4	1,013	5.7
398	8– 4–09	7.192	16.44	86	1,809	11.0	99	0.6	13,320	81.0	33	0.2	200	7.3
	3–11–10	6.712	8.22	80	1,085	13.2	126	2.5	6,520	79.3	90	1.1	271	3.3
	6–24–10	7.720	17.77	98	2,060	11.8	284	1.6	14,267	81.5	107	4.6	782	4.4
	3– 4–11	7.456	9.55	98	1,127	11.8	210	2.2	8,035	84.1	...	...	162	1.7
399	3– 9–09	7.080	18.66	85	4,666	25.0	1903	10.2	9,333	50.0	93	5.0	2,650	14.2
	4– 7–09	6.296	12.00	80	.....	....	....	....	......	....	...	...	.....	....
	8– 8–09	6.856	9.55	93	1,366	14.3	181	1.9	7,127	74.6	47	0.5	802	8.4
	3– 9–10	6.366	13.77	100	840	6.1	344	2.5	11,764	85.4	...	...	785	5.7
	5– 4–10	6.616	14.00	97	1,792	12.8	....	....	10,416	74.4	14	1.0	1,820	13.0
	6–24–10	5.992	19.11	93	.....	....	....	....	......	....	...	...	.....	....
	3– 4–11	5.728	10.55	98	1,161	11.1	116	1.1	8,791	83.3	31	0.3	369	3.5
400	3– 9–09	6.624	24.22	90	1,598	6.6	218	0.9	18,917	98.1	454	2.7	2,858	11.8
	4– 6–09	7.400	17.55	80	.....	....	....	....	......	....	...	...	.....	....
	8– 5–09	6.516	18.66	88	2,837	15.2	93	5.0	12,730	68.2	75	0.4	2,016	10.8
	3–28–10	5.928	11.44	85	1,259	11.0	526	4.6	8,468	74.0	46	0.4	915	8.0
	5– 4–10	3.776	14.00	87	1,176	8.4	280	2.0	11,088	79.2	56	0.4	1,456	10.4
	6–24–10	6.992	16.88	..	1,216	7.2	253	1.5	12,869	76.2	186	1.1	625	13.7
	3– 4–11	5.912	16.44	87	3,075	18.7	608	3.7	9,702	59.0	...	...	3,042	18.5

later six yearling heifers were introduced into the experiment. This completed the total number of animals to be examined. Table 5 gives the results for each individual for five or six different examinations extending over a period of four months. A differential count was also made and will be found in Table 7.

Table 6 includes further observations on the same animals and, in addition, shows the percentages of hemoglobin, and the number and percentage of the five different varieties of leukocytes. The observations over a period extending from March, 1909, to March, 1911.

In the following tables we have the observations made on the six calves introduced in the experiment two months after it was started. The period of experimentation extended from January 27, 1909 to March 11, 1911.

TABLE 7.

Number	Age in Years	Date	Reds	Whites	Polynuclears Number	Polynuclears Percentage	Mononuclears Number	Mononuclears Percentage	Lymphocytes Number	Lymphocytes Percentage	Mast Number	Mast Percentage	Eosinophiles Number	Eosinophiles Percentage
Grade Holstein 69–165	4/12	1–27–09	8.320	7.10	.....	....	.....	....	......	....	...	...	.....	...
		2–11–09	6.552	8.66	1,733	20.0	1,151	13.4	3,798	66.9	52	0.6	.....	...
		2–23–09	7.960	8.66	1,873	28.1	1,440	21.6	3,320	48.8	...	...	.....	...
		3–27–09	8.876	6.66	.....	....	.....	....	......	....	...	...	.....	...
		4–29–09	7.872	14.66	1,129	7.7	323	2.2	12,847	87.6	176	1.2	161	1.1
		8– 8–09	3.976	12.88	.....	....	.....	....	......	....	...	...	.....	...
		3–17–10	5.160	13.11	.....	....	.....	....	......	....	...	...	.....	...
		5–17–10	5.864	18.44	5,257	28.5	240	1.3	12,265	66.5	92	0.5	516	2.8
		6–12–10	5.288	16.00	2,032	12.7	480	3.0	12,688	79.3	112	0.7	730	4.5
		3–11–11	6.960	13.11	2,334	17.8	144	1.1	9,597	73.2	39	0.3	931	7.1
Grade Jersey 48–160	9/12	1–28–09	7.840	12.88	966	7.5	412	3.2	11,277	87.5	39	0.3	39	0.3
		2–12–09	9.408	16.66	.....	....	.....	....	......	....	...	...	.....	...
		2–24–09	8.848	18.88	.....	....	.....	....	......	....	...	...	.....	...
		3–22–09	9.728	22.00	5,544	25.2	1,078	4.9	14,960	68.0	154	0.7	198	0.9
		4–28–09	8.032	11.11	422	3.8	.....	....	10,555	95.0	111	1.0	22	0.2
		8– 6–09	5.420	6.22	.....	....	.....	....	......	....	...	...	.....	...
Grade Holstein 68–169	4/12	1–27–09	6.792	7.40	.....	....	.....	....	......	....	...	...	.....	...
		2–11–09	5.720	11.55	1,300	15.0	234	2.7	7,028	81.1	...	...	35	0.4
		2–23–09	6.088	11.33	.....	....	.....	....	......	....	...	...	.....	...
		3–22–09	6.488	11.11	844	7.6	800	7.2	9,455	85.1	...	...	.....	...
		4–28–09	5.912	11.11	2,233	20.1	.....	....	8,655	77.9	56	0.5	144	1.3
		8– 6–09	4.448	11.11	.....	....	.....	....	......	....	...	...	.....	...
		3–17–10	5.536	22.22	.....	....	.....	....	......	....	...	...	.....	...
		5– 7–10	6.648	15.11	1,723	11.4	363	2.4	11,438	75.7	166	1.1	1,390	9.2
		6–12–10	6.048	18.00	2,322	12.9	234	1.3	14,076	78.2	90	0.5	1,206	6.7
		3–11–11	5.440	16.88	2,837	16.8	203	1.2	12,834	76.0	68	0.4	946	5.6
Grade Jersey 47–167	4/12	1–28–09	8.000	5.00	No Records									
		2–12–09	7.160	8.22										
		2–23–09	5.864	9.77										
		3–27–09	7.008	10.00										
		4–29–09	7.960	12.00										
		8–1–09	Animal	died of tick fever.										

TABLE 7.—*Continued.*

Number	Age in Years	Date	Reds	Whites	Polynuclears Number	Polynuclears Percentage	Mononuclears Number	Mononuclears Percentage	Lymphocytes Number	Lymphocytes Percentage	Mast Number	Mast Percentage	Eosinophiles Number	Eosinophiles Percentage
		1-28-09	7.280	6.40	.....	....	...	...	......	....	...	...	.....	....
		2-12-09	7.656	8.00	832	10.4	432	5.4	6,696	83.7	...	...	40	0.6
		2-25-09	6.824	11.11	.....	....	...	...	......	....	...	...	.....	....
		3-24-09	6.516	7.33	.....	....	...	...	......	....	...	...	.....	....
Grade Jersey 19-170.......	$\frac{3}{12}$	4-28-09	7.248	8.00	2,422	30.9	...	...	5,512	68.9	8	0.1	8	0.1
		3-17-10	4.376	10.44	1,112	11.6	251	2.4	7,447	78.2	125	1.2	700	6.7
		5- 7-10	4.000	14.00	1,526	10.9	250	2.5	11,690	83.5	154	1.1	238	1.7
		6-12-10	5.288	12.00	1,608	13.4	337	2.8	9,792	81.6	...	...	240	2.0
		3- 7-11	5.384	12.66	241	19.0	431	3.4	9,500	75.0	...	...	304	2.4
		1-26-09	7.600	10.20	.....	....	...	...	......	....	...	...	.....	....
		2-11-09	5.870	8.44	.....	....	...	...	......	....	...	...	.....	....
		2-25-09	8.128	13.33	.....	....	...	...	......	....	...	...	.....	....
		3-24-09	6.484	9.33	1,288	13.8	233	2.5	7,792	83.5	...	...	.....	....
Grade Holstein 67-178.......	$\frac{3}{12}$	4-28-09	8.968	8.66	.....	....	...	...	......	....	...	...	.....	....
		8-,6-09	4.352	10.00	.....	....	...	...	......	....	...	...	.....	....
		3-17-09	6.272	16.00	1,984	12.4	400	2.5	12,432	77.7	80	0.5	1,072	6.7
		5- 7-10	5.320	9.33	.....	....	...	...	......	....	...	...	.....	....
		6-12-10	5.728	12.88	1,688	13.1	361	2.8	9,331	72.4	128	1.0	1,353	10.5

TABLE 8.

GENERAL AVERAGES OF REDS, WHITES, DIFFERENTIAL COUNTS OF WHITES AND HEMOGLOBIN.

NUMBER	REDS	WHITES	PERCENTAGE OF HEMOGLOBIN	POLYNUCLEARS		MONONUCLEARS		LYMPHOCYTES		MAST		EOSINOPHILES	
				Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
382......	6.896	11.43	90	1,612	14.1	377	3.3	8,735	76.4	69	0.6	938	8.2
383......	4.720	20.64	83	1,486	7.2	536	2.6	17,172	83.2	123	0.6	1,382	6.7
384......	6.032	9.75	81	1,912	19.6	244	2.5	6,801	69.6	78	0.8	2,077	12.2
385......	5.708	11.92	81	1,550	13.0	357	3.0	8,791	73.7	71	0.6	1,216	10.2
393......	5.360	12.33	85	974	7.9	210	1.7	10,397	84.3	101	0.9	654	5.3
397......	5.180	19.97	85	2,018	10.1	439	2.2	16,360	81.9	120	0.6	1,019	5.1
398......	7.270	12.99	90	1,547	11.9	221	1.7	10,580	81.4	78	0.6	533	4.1
399......	6.419	13.95	92	1,925	13.8	544	3.9	13,044	93.5	70	0.5	1,242	8.9
400......	6.164	17.03	86	1,907	11.2	494	2.9	12,330	72.4	136	0.8	2,077	12.2

TABLE 9.

General average of six calves sampled between the dates of January 27, 1909, and March 11, 1911.

Reds. 6,742,000
Whites. 11,632

Differential Count of Leukocytes of the Above Six Calves.

	Per c.c.	Average per cent	Minimum per cent	Maximum per cent	
Lymphocytes.	9,740	77.8	49.8	95.0	Personal observations
Mononuclears.	452	3.8	0.6	21.6	
Polynuclears.	1,873	14.6	3.8	30.9	
Eosins.	524	3.9	0.1	10.5	
Mast.	92	0.6	0.1	1.2	

Before the work represented in the above tables was completed it was seen that the results in some respects were different from those obtained by other workers in this line. It seemed necessary, therefore, to determine if possible the cause of this difference, so a number of animals in the general herd living under supposedly normal conditions were tested. In Table 10 we have the results obtained from 41 individual cattle.

TABLE 10.

Name	Breed	Date	Age in Years	Reds	Whites	Percentage of Hemoglobin	Polynuclears		Mononuclears		Lymphocytes		Mast		Eosinophiles	
							Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Boyd	P. B. J.	3-13-10	2	6,440,000	11,776	70	.....	....	...	...	......	....	...	...	.....	....
86	G. J.	3-31-10	3	5,568,000	14,444	90	.....	....	...	...	......	....	...	...	.....	....
Fair Tabby	P. B. J.	6- 2-10	13	....	......	..	.....	6.1	...	0.7	......	84.2	...	...	.....	8.8
Bettie's Hef	P. B. R. P.	6-15-09	6	7,816,000	26,222	..	1,075	4.1	...	...	24,622	93.9	184	0.7	288	1.1
171	G. J.	1-15-09	4	6,464,000	12,444	89	983	7.9	...	...	10,615	85.3	25	0.2	513	4.2
R. Sanders	G. R. P.	6-16-09	9	5,980,000	18,444	83	738	4.0	...	...	14,117	76.0	221	1.2	3,320	18.0
Meslova's Pet.	P. B. R. P.	6-16-09	6	5,464,000	11,554	88	2,622	14.9	...	...	8,514	73.7	58	0.5	1,433	11.4
Maud	P. B. R. P.	6-16-09	7	6,704,000	10,222	88	838	8.2	...	...	8,709	85.2	51	0.5	593	5.8
230	G. J.	3-23-10	8	6,208,000	8,444	94	1,266	15.0	228	2.7	6,519	77.2	...	...	355	4.2
92	G. J.	3-23-10	3	5,200,000	16,888	91	2,702	16.0	540	3.2	11,720	69.4	34	0.2	2,027	12.0
330	G. J.	3-23-10	6	6.720,000	10,888	98	1,753	16.1	217	2.0	8,493	78.0	...	...	403	3.7
203	G. J.	3-23-10	2	5,832,000	12,220	95	709	5.8	244	2.0	10,069	82.4	73	0.6	1,124	9.2
84	G. J.	3-31-10	3	5,784,000	25,776	90	5,052	19.6	851	3.3	17,889	69.4	103	0.4	3,248	12.6
364	G. J.	3-31-10	3	5,400,000	19,110	93	2,216	11.6	573	3.0	14,523	76.0	191	1.0	1,529	8.0
Turner	P. B. R. P.	6-15-09	5	6,560,000	14,444	88	1,632	11.3	...	...	12,306	85.2	...	...	404	2.8
Butts 85	G. J.	6-15-09	5	5,880,000	16,000	87	544	3.4	...	...	14,560	91.0	192	1.2	576	3.6
91	G. J.	6-14-09	3	6,600,000	27,110	90	2,006	7.4	...	...	23,803	87.8	81	0.3	1,166	4.3
20	G. J.	6-14-09	10	5,912,000	13,110	81	2,910	22.2	...	...	8,535	65.1	249	1.9	1,390	10.6
21	G. J.	6-14-09	8	5,592,000	4,888	81	440	9.0	...	...	3,612	73.9	34	0.7	391	8.0
39	G. J.	6-14-09	8	6,392,000	11,322	85	850	7.5	...	...	9,077	80.1	34	0.3	1,213	10.7
111	G. J.	6- 7-09	7	5,848,000	10,222	81	951	9.3	...	...	8,177	80.0	51	0.5	1,022	10.0
78	G. J.	6- 7-09	3	6,888,000	14,000	80	2,576	18.4	...	...	10,388	74.2	98	0.7	882	6.3
100	G. J.	7- 5-10	2	4,984,000	8,000	50	832	10.4	208	2.6	6,296	78.7	32	0.4	624	7.8
Evelene	P. B. J.	7- 5-10	4	5,576,000	6,632	85	610	9.2	206	3.1	5,306	80.0	40	0.6	491	7.4
166	G. J.	7- 5-10	3	5,280,000	11,666	91	1,248	10.7	455	3.9	8,400	72.0	23	0.2	960	8.4
Lura K.	P. B. J.	7- 5-10	4	5,792,000	12,554	70	1,720	13.7	615	4.9	9,252	73.7	...	...	942	7.5
Timola's Lassie.	P. B. J.	7- 5-10	6	5,600,000	7,666	70	958	12.5	352	4.6	5,823	76.1	...	...	521	6.8
225	P. B. J.	6- 2-10	2	6,248,000	9,544	65	1,116	11.7	67	0.7	7,511	78.7	29	0.3	773	8.2
66	G. J.	6- 7-09	6	5,616,000	12,666	85	924	7.3	190	1.5	10,348	81.7	...	...	1,165	9.2
272	G. J.	6- 7-09	7	6,056,000	7,776	84	544	7.0	...	...	5,913	77.6	69	0.9	1,111	14.3
18	G. J.	4-21-09	12	5,400,000	8,554	83	2,395	28.0	...	...	5,560	65.0	85	1.0	718	8.4
103	G. J.	4-21-09	10	6,336,000	4,444	81	848	19.1	...	...	3,218	74.9	...	...	182	4.1
202	G. J.	4-13-09	8	6,976,000	8,000	86	1,248	15.6	...	...	5,856	73.2	48	0.6	848	10.6
119	G. J.	4-12-09	2	5,320,000	11,110	..	911	8.2	533	4.8	9,243	83.2	44	0.4	377	3.4
118	G. J.	4-12-09	2	4,504,000	6,732	78	1,252	18.6	...	...	4,712	70.0	94	1.4	673	10.0
104	G. J.	6- 4-09	7	6,392.000	15,332	88	1,594	10.4	...	...	12,909	84.2	30	0.2	720	4.7
Estelle	P. B. J.	6- 2-10	6	5,576,000	12,000	90	864	7.2	288	2.4	10,152	84.6	...	...	60	5.0
Bettie	P. B. R. P.	7-19-10	5	7,104,000	8.222	88	2,006	24.4	65	0.8	5,688	70.4	...	...	353	4.2
174	G. J.	3-23-10	8	7,608,000	7,666	96	1,303	17.0	...	...	5,496	71.7	...	...	850	11.1
Roxie L.	P. B. J.	6- 2-10	5	5,592,000	11,998	89	2,052	17.1	131	1.1	8,326	69.4	35	0.3	1,403	11.7
Maimie L.	P. B. J.	6- 2-10	10	6,000,000	11,110	70	955	8.5	133	1.2	8,699	78.3	...	...	1,344	12.1
Average of 41 cases		....	5.7	6,053,600	12,361	83	.....	11.7	...	2.5	......	77.9	...	0.6	.....	7.8
Average of 15 cases		....	...	6,338,000	14,080	85	.....	12.0	...	2.6	......	79.6	...	0.7	.....	7.6

NOTE.—P. B. J. = Pure Bred Jersey; G. J. = Grade Jersey; P. B. R. P. = Pure Bred Red Poll; G. R. P. = Grade Red Poll.

Comparison of Results.

General average of nine cows sampled between the dates of October 22, 1908, and April 8, 1909. These were on a feeding experiment.

Reds	6,704,000
Whites	13,712

General average of nine cows sampled between the dates of March 9, 1909, and March 4, 1911. These were on a feeding experiment.

Reds	5,972,000
Whites	14,449
Hemoglobin	85 per cent

Average of the above results.

Reds	6,338,000
Whites	14,080
Hemoglobin	85 per cent

Average of 41 cows in the general herd. These were not in any experiment.

Reds	6,053,600
Whites	12,361
Hemoglobin	83.7 per cent

Red Corpuscles per c.c.	Leukocytes per c.c.	Hemoglobin per cent	Authors
6,152,000	5,486	59.7	Dimock and Thompson
6,000,000	9,730	....	Smith and Kilbourne
5,473,000	8,241	....	Storch
6,053,600	12,361	83.7	Personal observations (41 cases)

Differential Count of Leukocytes.

	Per c.c.	Average per cent	Minimum per cent	Maximum per cent	
Lymphocytes	2,992	54.2	31.0	76.0	Dimock and Thompson
Large mononuclears	86	21.4	20.2	3.3	
Polymorphonuclears	1,786	30.5	13.0	45.8	
Eosins	772	13.15	3.8	26.5	
Mast	31	0.59	0.1	1.2	

Results of Observation on 41 Cows in General Herd.

	Per c.c.	Average per cent	Minimum per cent	Maximum per cent	
Lymphocytes	9,568	79.9	65.0	93.9	Personal observations
Large mononuclears	327	2.5	0.7	4.9	
Polymorphonuclears	1,820	11.7	3.4	28.0	
Eosins	1,005	7.8	1.1	18.0	
Mast	80	0.7	0.2	1.9	

Results of Observation on Nine in a Feeding Experiment.

	Per c.c.	Average per cent	Minimum per cent	Maximum per cent	
Lymphocytes	12,283	79.6	50.0	92.1	
Large mononuclears	368	2.6	0.4	10.2	
Polymorphonuclears	1,620	12.0	3.2	30.6	Personal observations
Eosins	963	7.6	1.4	18.5	
Mast	98	0.7	0.2	2.7	

A glance at the preceding tables will reveal some differences when compared with the already accepted data of other observers. At first the records were thought to be faulty though the methods used were the same then as now. The work was done then with as great care as at the present time. The work was repeated and allowed to extend over long periods. The results were, in general, the same as at first. No reason could be given to account for the differences. The idea, however, that the Texas fever parasite must exert some influence persistently suggested itself. In order to see if there was any foundation for this belief five yearling heifers which had just been imported from Pennsylvania were examined and a normal established. Afterward these same animals were inoculated with Texas fever organisms. One died within the prescribed 10 days and within 15 minutes of the time of death the blood was examined. The other animals all survived the treatment and after three later attacks the blood was sampled at intervals as shown in the table below. No definite conclusions should rightly be made, since the number of cases are few, but the indications strengthen the belief that the differences between our observations and those of other workers are traceable to the Texas fever protozoon.

The following tables include further observations on the same animals, and, in addition, show the percentage of hemoglobin and the number and percentage of the five different varieties of leukocytes.

The results of the tables can be more strikingly arranged in the following manner: The animals had recovered from three attacks and had been passed on as in good condition a month before the clinical examination represented in the second column. The

TABLE II.

Normal (before Treatment with Texas Fever Organisms).

Name of Animal	Date	Reds	Whites	Percentage of Hemoglobin	Polynuclears		Mononuclears		Lymphocytes		Mast		Eosinophiles	
					Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Daisy	7–13–10	7,424,000	9,440	98	1,133	12.0	283	3.0	7,779	82.4	38	0.4	264	2.8
Megs of Hyland	7–13–10	6,992,000	10,666	96	1,920	18.0	107	1.0	8,127	76.2	..	...	405	3.8
Yearling	7–13–10	7,656,000	6,222	95	759	12.2	75	1.2	5,251	84.4	37	0.6	100	1.6
Lady Monkland	7–13–10	5,208,000	10,666	100	2,027	19.0	245	2.3	8,202	76.9	..	...	181	1.7
Heifer No. 30	7–13–10	7,872,000	7,766	97	1,328	17.1	148	1.9	5,957	76.7	..	...	318	4.1
Average		7,030,000	8,950	97.2	1,433	15.6	171	1.88	7,163	79.3	37	0.5	253	2.8
The following counts were taken after three attacks, Heifer No. 30 having died after the first attack.														
Daisy	11–18–10	5,056,000	19,554	88	4,302	22.0	1,036	5.3	14,079	72.0	..	...	39	0.2
Megs of Hyland	11–18–10	5,872,000	43,332	95	2,947	6.8	1,430	3.3	37,916	87.5	43	0.1	823	1.9
Yearling	11–18–10	4,960,000	14,444	84	303	2.1	419	2.9	13,606	94.2	..	...	101	0.7
Lady Monkland	11–18–10	5,040,000	20,666	95	1,994	9.6	847	4.1	15,520	75.1	62	0.3	1,384	9.6
Average		5,233,000	24,499	90	2,384	10.2	383	3.9	20,280	82.2	52	0.2	586	3.1
Daisy	12– 1–10	3,968,000	18,444	88	1,420	7.7	1,107	6.0	14,811	80.3	..	...	1,070	5.8
Megs of Hyland	12– 1–10	5,584,000	30,888	84	1,820	5.9	710	2.3	26,934	87.2	..	...	1,390	4.5
Yearling	12– 1–10	5,288,000	16,332	84	1,633	10.0	294	1.8	14,258	87.3	..	...	131	0.8
Lady Monkland	12– 1–10	6,408,000	25,554	89	4,370	17.1	690	2.7	20,009	78.3	..	...	434	1.7
Average		5,312,000	22,804	86	2,310	10.2	700	3.2	19,003	83.2	..	...	756	3.2
Daisy	1–31–11	7,040,000	32,000	85	4,608	14.4	.....	...	25,120	78.5	..	...	2,272	7.1
Megs of Hyland	1–31–11	7,840,000	28,888	94	4,333	15.0	433	1.5	22,677	78.5	87	0.3	1,329	4.6
Yearling	1–31–11	7,488,000	14,888	84	2,680	18.0	462	3.1	11,464	77.0	..	...	253	1.7
Lady Monkland	1–31–11	6,432,000	16,444	83	2,154	13.1	181	1.1	13,711	77.3	..	...	1,332	8.1
Average		7,200,000	23,055	86.5	3,444	15.1	358	1.9	18,243	77.8	87	0.3	1,296	5.3
NOTE.—Heifer No. 30 died on the 22d of July. 15 minutes afterward an examination was made with the following results. A sample just before death would have been more reliable and therefore, preferable, but unfortunately we were informed too late.														
Heifer No. 30	7–22–10	2,192,000	31,332	32	.....	41.4	.....	2.9	......	53.4	..	...	.....	....
Daisy	4–14–11	7,440,000	31,554	90	4,102	13.0	726	2.3	23,445	74.3	95	0.3	3,092	9.8
Megs of Hyland	4–14–11	5,704,000	15,110	80	2,418	16.0	212	1.4	11,060	74.2	..	...	1,420	9.4
Yearling	4–14–11	6,672,000	19,554	95	3,050	15.6	704	3.6	13,766	70.4	..	...	2,034	10.4
Lady Monkland	4–14–11	6,760,000	20,666	89	2,144	10.4	434	2.1	15,706	76.0	41	0.2	2,315	11.2
Average		6,644,000	21,721	88.5	2,930	14.0	519	2.3	15,994	73.4	68	0.25	2,215	10.2

second examination was made on the 1st of December, the third on the 31st of January, and the last on the 14th of April.

We note that there was a decrease in reds still but that the number was gradually approaching the normal. The percentage of hemoglobin also decreased, while the number of leukocytes increased beyond the normal and was continuing to increase.

TABLE 12.

OBSERVATIONS OF FIVE HEIFERS.

General Results.

	Before Treatment	After Treatment			
		Date 11-18-10	Date 12-1-10	Date 1-3-11	Date 4-14-11
Reds	7,030,000	5,233,000	5,312,000	7,200,000	6,644,000
Whites	8,950	24,444	22,804	23,005	21,721
Percentage of Hemoglobin	97	90	86	86.5	88.5

Differential Count of Whites.

	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Polynuclears	1,433	15.6	2,384	10.2	2,310	10.2	3,444	15.0	2,930	14.0
Mononuclears	171	1.88	383	3.9	700	3.2	858	1.9	519	2.3
Lymphocytes	7,163	79.3	20,280	82.2	19,003	83.2	18,243	77.8	15,994	73.4
Mast	37	.5	52	.2	......	....	87	.3	68	.2
Eosinophiles	253	2.8	586	3.1	756	3.2	1,296	5.3	2,215	10.2

A glance at the table will reveal an increase in the percentage of lymphocytes, eosinophiles, and mononuclears and a decrease in the percentage of polynuclears and mast cells.

CONCLUSIONS.

Our original observations on 15 animals in the dairy herd gave us results which, when compared with those of other observers, showed some marked differences. The most apparent differences are noted as follows: (1) The number of whites is appreciably larger. (2) The number and percentage of polynuclears are smaller. (3) The number and percentage of lymphocytes are larger. Other differences, though not so apparent and possibly of very little value, are noted as follows: (1) The number and percentage of mast cells have decreased, while the number and percentage of eosinophiles and mononuclears have increased.

These same differences were observed when the above 15 animals were further examined at various times and over a period of two years. When the results of the original 15 animals were compared with the results of a set of 15 animals from the general herd, the same differences were also noted. Furthermore, we observed similar differences when we examined 41 cows in the general herd.

To account for these differences was our next object, for we did not consider that we had established a new normal. The idea that tick fever might have such an effect on the blood of the animal that had passed through one or more attacks persistently suggested itself; so when five Ayrshire heifers were imported from Pennsylvania, observations were immediately made to establish a normal. After these observations the animals were put through a system of immunization to tick fever. A month after they had passed through three attacks and were considered in good condition, the blood was examined at four different times. These results were found to be similar to our other results. The number of cases (four) is small, and though too small to draw definite conclusions from, we believe that we have sufficient indication to justify our position and hope on further observation to establish this fact without a doubt.

One animal died and observations 15 minutes after death showed an increase of whites. We have found this to be true in three other cases. It is hoped that time and material will be available for a more thorough study of the leukocytes in the last stages of the disease.

THE INTERPRETATION OF TESTS FOR B. COLI COMMUNIS.*

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The presence of *B. coli communis* in water is assumed to indicate the presence of sewage pollution and typhoid germs.

Several species of bacteria resemble the colon bacillus, and if we are going to regard the presence of *B. coli communis* as the criterion of pollution, it is important to have an accurate method for its identification, and, as the operation of water purification works is modified as the numbers of *B. coli* increase or decrease it is also important that the method be rapid.

At the present time the fermentation of sugar solutions offers the readiest means for identification. This method has been used for years, but some new suggestions made by Mr. D. D. Jackson[1] in regard to the use of dulcite, raffinose, and mannite looked so promising that a series of comparative tests in various media have been carried out at the Columbus Water Purification Works. Tests were made on many duplicate cultures of bacteria isolated from the river and purified water at the plant, and upon duplicate cultures obtained from nine different laboratories. Summaries of these results will be given in the following pages.

In our daily routine analysis of the river and filtered water for the presence of *B. coli* it has been our custom to transplant cultures from an agar slant into lactose bile, and if gas develops the culture is then transplanted into milk, nitrate solution, Dunham's peptone solution for indol, and gelatin (as outlined in "Standard Methods"). We found that, out of a total of 3,000 cultures, about 65 per cent of the lactose-bile fermenting organisms gave positive tests in these media; 28 per cent failed to produce indol; 5 per cent did not reduce nitrate; and there were some that liquefied gelatin.

* Received for publication April 25, 1911.

[1] *Jour. Infect. Dis.*, 1911, 8, p. 241.

At the end of each month for two or three months, a number of cultures were chosen from those tested during the month. These cultures were purified and revivified according to the standard method and were transplanted in duplicate into lactose bile, dextrose broth, saccharose broth, dulcite broth, Dunham's solution for indol, nitrate solution, gelatin, milk, neutral red dextrose broth, Endo medium, dextrose broth to which NaOH was added after incubation for the presence or absence of red color, and esculin bile solution.

The different strains of lactose-bile fermenting organisms that we have isolated, by the use of these media, are shown in Table 1.

TABLE 1.

	Gas in Dulcite	Gas in Saccharose	Indol	Nitrate Reduced	No. of Days to Blacken Esculin Bile Solution	NaOH Red Test in Dextrose
Culture 1	o	o	o	+	10	o
" 2	o	o	+	+	10	o
" 3	+	o	+	o	8	o
" 4	+	o	o	+	10	o
" 5	+	o	+	+	2	o
" 6	o	+	o	+	10	o
" 7	o	+	+	+	10	+
" 8	+	+	o	+	4	+
" 9	+	+	o	+	1	+

These organisms all ferment dextrose and lactose, coagulate milk, give positive reactions on Endo medium and in neutral red broth, and do not liquefy gelatin.

These different lactose-bile fermenting organisms are non-chromogenic, but we have isolated two organisms that give gas in lactose bile that are easily separated from the group by the fact that one produces a brown pigment and the other a yellow. These seem to be rather common in our raw water supply, so that the bile presumptive test for *B. coli* would often be misleading.

TABLE 2.

Culture	Gas in Dulcite	Indol	Nitrate
1	o	o	+
2	o	+	+
3	+	+	o
4	+	o	+
5	+	+	+

It will be noticed that these cultures may be divided into two classes on the basis of the positive and negative fermentation of

saccharose. Cultures 1, 2, 3, 4, and 5 do not ferment saccharose, and their culture differences may be shown in Table 2.

Cultures 6, 7, 8, and 9 ferment saccharose, and their cultural differences are shown in Table 3.

TABLE 3.

Culture	Gas in Dulcite	Indol	NaOH Red Test	More than 24 Hrs. to Blacken Esculin
6	o	o	o	+
7	o	+	+	+
8	+	o	+	+
9	+	o	+	o

The lactose-bile fermenting organisms obtained from outside laboratories were also run in duplicate in all the above-named media and also in raffinose and mannite. The results are shown in Table 4.

TABLE 4.

Cultures from Outside Laboratories	Gas in Dulcite	Gas in Saccharose	Gas in Mannite	Gas in Raffinose	Indol	Gelatin Liquefied	No. of Days Required to Blacken Esculin	NaOH Red Test in Dextrose
B. coli 1	+	—	+	+	+	o	4	o
" " 2	+	—	+	+	+	o	4	o
" " 3	+	o	+	o	+	o	2	o
" " 4	+	o	+	o	+	o	3	o
" " 5	+	o	+	o	+	o	3	o
" " 6	+	o	+	o	+	o	2	o
" " 7	+	o	+	o	+	o	2	o
" " 8	o	o	+	o	+	o	3	o
" " 9	+	o	+	o	+	o	3	o
" " 10	+	+	+	+	+	o	8	o
" " 11	+	+	+	+	+	o	5	o
" " 12	+	o	+	o	+	o	11	o
" " 13	—	o	+	+	+	o	2	o
" " 14	—	+	+	+	+	o	5	o
" " 15	—	+	+	+	+	o	5	o
B. coli communior A	+	+	+	+	o	o	—	+
B. aerogenes 1	—	+	—	—	+	o	3	o
B. aerogenes 2	+	+	+	+	+	o	8	o
B. lactis aerogenes	+	+	+	+	o	o	1	+
B. cloacae	+	+	+	+	o	+	5	+
B. aerogenes capsulatus	—	+	—	—	o	+	—	+
B. acidi lactici	o	o	+	o	+	o	o	o

These cultures all ferment dextrose and lactose, coagulate milk, reduce nitrate, and give positive reactions on Endo medium and in neutral red broth.

The esculin bile solution used in this work was made according to the following formula.[1]

[1] Harrison and Vanderleck, *Centralbl. f. Bakt.*, Abt. 1, *Orig.* 1910, 51, p. 607.

1 per cent Witte's peptone
0.5 per cent sodium taurocholate (commercial)
0.1 per cent esculin
0.05 per cent ferric citrate
100 c.c. tap water.

After steaming 15 to 20 minutes the medium is filtered, tubed, and sterilized (fractional sterilization).

It will be noticed in the table that the time necessary to change the color of this solution from a clayish color to a jet black ranges from less than one day to 11 days. *B. lactis aerogenes* is the only lactose-bile fermenting bacterium that we have been able to isolate that produces the black color in less than one day. *B. coli communis* requires two to three days, and some of the other forms require a much longer time.

Another thing to be noticed is the close similarity of *B. coli communis*, *B. acidi lactici*, *B. aerogenes*, and *B. coli communior*. By the standard confirmatory tests these four strains of lactose-bile fermenting organisms would all be called *B. coli*.

The value of saccharose and dulcite broth as a differential test for these strains is shown in Table 5.

TABLE 5.

Species	Gas in Saccharose	Gas in Dulcite
B. coli communior	+	+
B. aerogenes	+	+
B. coli communis	o	+
B. acidi lactici	o	o

The different organisms that we have studied may be grouped into two classes: (1) those that ferment saccharose; (2) those that do not ferment saccharose.

Non-saccharose fermenters: *B. coli communis* and *B. acidi lactici*. Saccharose fermenters: *B. aerogenes*, *B. coli communior*, *B. lactis aerogenes*, *B. aerogenes capsulatus*, and *B. cloacae*.

B. coli is differentiated from *B. acidi lactici* by one (*B. coli*) fermenting dulcite and the other (*B. acidi lactici*) not fermenting dulcite.

The five organisms listed under the heading saccharose fermenters all produce gas from the various sugars; however, not to

the same extent. *B. lactis aerogenes* produces from 80 to 100 per cent gas in both lactose bile and dextrose broth. *B. aerogenes capsulatus* produces much gas in bile (75 to 90 per cent) but only about 25 to 30 per cent in dextrose. *B. cloacae* only gives about 15 per cent gas in lactose bile. The other cultures range from 35 to 60 per cent gas in 48 hours. The most striking cultural characteristics of the organisms in this class are shown in Table 6.

TABLE 6.

Species	Gelatin Liquefied	Indol	More than 24 Hrs. to Blacken Esculin	NaOH Red Reaction
B. aerogenes....................	o	+	+	o
B. communior..................	o	o	+	+
B. lactis aerogenes..............	o	o	o	+
B. aerogenes capsulatus..........	+	o	+	+
B. cloacae.....................	+	o	+	+

It will be noticed that *B. aerogenes capsulatus* and *B. cloacae* separate themselves from the group by being gelatin liquefiers. *B. aerogenes* produces indol in Dunham's solution and *B. lactis aerogenes* and *B. communior* do not. *B. lactis aerogenes* can be identified by the very quick aesculin reaction.

It will be seen from the foregoing tables that other bacteria besides *B. coli communis* will produce gas in lactose bile, and we believe that in the differentiation of this organism the purified cultures that give gas in lactose bile should be next transplanted into saccharose. If the culture fails to produce gas in saccharose then subcultures should be made into indol and nitrate broths, and if both show positive reactions then transplant into dulcite. If this reaction is positive call the strain *B. coli communis.* Those organisms that depart from the above tests may be called lactose-bile fermenting organisms, not *B. coli communis*, or may be classified as belonging to the colon group, or they may be run down to positive identification.

The principal differences observed in the organisms that we have studied in this laboratory are shown in the diagnostic table (Table 7).

The table is a résumé of the most illuminating cultural characteristics shown by the different lactose-bile fermenting organisms and

TABLE 7.

Species	Sac-charose	Dulcite	Gelatin Liquefied	Indol	Nitrate	Esculin Days to Blacken More than One
(A) Unknown	o	o	o	o	+	+
(B) Unknown	o	o	o	+	o	+
B. acidi lactici	o	o	o	+	+	+
(C) Unknown	o	+	o	o	+	+
B. coli communis	o	+	o	+	+	+
(D) Unknown	+	o	o	o	+	+
(E) Unknown	+	o	o	+	+	+
B. communior	+	+	o	o	+	+
B. lactis aerogenes	+	+	o	o	+	o
B. cloacae	+	+	+	o	+	+
B. aerogenes cap.	+	−	+	o	+	+
B. aerogenes	+	o	o	+	+	+

it will be noticed that according to the differential tests that have been adopted as standards, we do not differentiate between *B. coli communis* and at least three other distinct species. In the diagnostic scheme given for the identification of *B. coli communis* it is well to carry out the tests in the order mentioned because it saves work and media, and as the dulcite is expensive it is best to select very small fermentation tubes, and only use this medium to differentiate between *B. acidi lactici* and *B. coli communis.*

In the daily routine analysis of the filtered water for the presence of *B. coli communis* it is our practice to proceed according to the following method.

1. Pour azolitmin lactose Parietti agar plates using 1 c.c. of sample.
2. Inoculate 1 c.c. portion of sample into dextrose broth.
3. Inoculate 1 c.c. portion of sample into lactose bile.
4. Inoculate 50 c.c. portion of sample into 10 c.c. of 10 times normal strength broth.
5. Incubate at 37° to 40° C.
6. At the end of 24 hours transplant 1 c.c. from the 50 c.c. enriched sample into lactose bile.
7. Pour plates from all fermentation tubes that show gas.
8. Fish three characteristic colonies (if present) on to agar slopes.
9. Transplant from agar slope in lactose bile.
10. If no gas develops make a negative report.
11. If gas develops then transplant from agar slope into saccharose broth, and if gas develops classify as belonging to *B. coli* group or lactose-bile fermenting organisms not *B. coli communis.*
12. If no gas develops continue the subculturing into nitrate and indol broths; if these are both positive we regard the bacterium as either *B. coli communis* or *B. acidi lactici.*
13. Subculture into dulcite; if positive gas production, record as *B. coli communis.*

Our records for the bacterial analysis of the purified water are kept on a printed sheet as shown below.

FILTERED WATER, DATE..........1911
Time..........M.

No. of Bacteria per c.c. at			Dextrose Broth 1 c.c.		Lactose Bile 1 c.c.	Azolitmin Lactose Agar Plates		50 c.c. Enriched 1 c.c. into Bile
20° C.	37° C.	Gelatin	Turb.	Per cent Gas	Per cent Gas	Poured	Colonies Fished	Per cent Gas

		Gas in Bile	Gas in Sacc.	Nitrate	Indol	Gas in Dulcite	B. coli communis	B. coli Group
Cultures from Blue Plates	1							
	2							
	3							
Cultures from Broth	I							
	II							
	III							
Cultures from Bile	A							
	B							
	C							
Cultures from 50 c.c. Portion	a							
	b							
	c							

Remarks

B. coli is found in sewage and therefore its presence is significant; but as newspaper reporters and other persons are inclined to magnify conditions, the officers in charge of purification works should be careful to identify suspicious organisms as true *B. coli* before throwing suspicion upon their plants and prejudicing the minds of the people. With this end in view the procedure outlined in the diagnostic table places a ready method of proving whether waters do or do not contain *B. coli communis*, and the necessity of such procedure is illustrated by the variety of organisms which less thorough tests would include erroneously in the group.

EXPERIMENTAL IMMUNITY WITH REFERENCE TO THE LEPROSY BACILLUS.*

PART II.†

A STUDY OF THE FACTORS DETERMINING THE CURE OF INDIVIDUALS INFECTED BY THE BACILLUS LEPRAE.

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The foundation for the horror and fear in which leprosy is held by the laity and also to a less marked degree by the medical profession depends chiefly on the popular belief in the absolute incurability of the disease. If it be possible to remove from leprosy the stigma of incurability and the corollary of ultimate fatality the disease will be relegated to a much less important place in sociologic, economic, and medical literature than tuberculosis and syphilis. Theoretically, it is difficult to conceive of any bacterial disease being incurable; there are, too, on record a sufficient number of well-attested cases of undoubted leprosy which have recovered and remained well to prove that under certain conditions cure of the disease may take place. Isadore Dyer[1] among others has published records of a number of cases where the infection has been eradicated by our present methods of treatment and regimen.

Among animals susceptible to both local and generalized lesions there is undoubtedly present some mechanism of defense, as the result of which the bacilli are unable to live indefinitely in their tissues. The protocols of monkeys, mice, goats, and guinea-pigs which have shown, a few months after a more or less fulminant infection, absolutely no signs of the disease either macroscopically or microscopically could be cited as evidence of the capacity of the animal tissues to eradicate the bacilli.

During the past eighteen months we have carried out several series of experiments in the hope of finding some clue which might

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† Part I appeared in the *Jour. Exper. Med.*, 1911, 14, p. 181.

[1] *Medical News*, 1905, 68, p. 230.

lead to the discovery of the manner in which the protective substances act and what they are, and, if possible, of procuring some means for increasing the potency and rapidity of development of such bodies as might be employed in the treatment of human leprosy. We have demonstrated[1] that the ordinary antibodies, such as agglutinins, amboceptors, etc., may be induced in animals by the injection of dead bacilli. In no instance, however, has it been possible to produce these bodies in similar proportions to those developed experimentally with many other bacteria nor has it been possible to protect animals against subsequent infection by means of preliminary injections of the dead organisms.

As stated in a former publication,[2] most animals, though more or less readily infected with leprosy bacilli, recover in a comparatively short time even when the percentage of antibodies present in their serum at the time of recovery is low. Such animals, moreover, are susceptible to reinfection after their recovery from a primary infection; this observation suggests that some mechanism other than the commonly considered immune bodies is active in the cure of the disease. It appears, therefore, that among animals the cause of the recovery from infection is rather the result of the inability of the bacillus to prolong its existence in the host than due to the development of any active protective process on the part of the infected animal. It is reasonable to suppose that protection is afforded the microorganism by the proliferation of cells of the epithelioid type and that with the disappearance of such cells and the exposure of the bacilli to the action of the tissue juices they are destroyed.

The results of our experiments demonstrate that although under ordinary conditions the leprosy bacillus is able to prolong indefinitely its existence in the human tissues, it is possible for the polymorphonuclear leukocytes to digest and destroy it. Normally, however, the pus cells only infrequently attack the organisms. The alteration in conditions necessary to bring about such reaction appears to be one affecting the bacteria themselves. We do not believe that this change is of the nature of an opsonic reaction, but rather is the result of the bacilli becoming more toxic and thus

[1] *Jour. Exper. Med.*, 1911, 14, p. 100.

[2] *Loc. cit.*

stimulating polymorphonuclear activity. This increased toxicity seems to be due to a hypersensitiveness of the individual which results in an anaphylactic toxicity.

Although animals infected with the leprosy bacillus usually recover spontaneously in a comparatively short time and for this reason it is practically useless to attempt to test the therapeutic value of any procedure upon them, certain observations are of sufficient interest and importance to justify mention here.

Normal monkeys and guinea-pigs, when inoculated subcutaneously with living or dead leprosy bacilli, either do not react or show merely the development of a small area of hyperemia and edema accompanied by a proliferation of lymphoid, plasma, and epithelioid cells. As mentioned in a former publication,[1] there usually develops in animals previously sensitized to the bacilli a more extensive lesion which persists over a longer period and shows, if viable bacilli have been employed, a greater tendency to metastasis. In a certain percentage of animals, however, and this has been noted more especially in two monkeys which had recovered from a severe leprosy infection, an interesting and important phenomenon was observed. The reaction in these animals occurred in the following manner. Within 24 hours after the inoculation of one billion dead bacilli, there developed at the site of inoculation a somewhat reddened and apparently painful swelling which gradually increased in size, became softer, and about the third to fifth day fluctuated. Microscopically, the content of the mass was composed almost entirely of polymorphonuclear leukocytes, many of which had taken up the leprosy bacilli.

Among other animals we have observed similar reactions following second or third inoculations. This was especially noted in a horse inoculated in an effort to procure an antiserum. This animal received a primary dose of approximately two billion dead bacilli. Four days after the injection there developed at the site of inoculation a firm, raised mass nine centimeters in diameter, which gradually subsided and had practically disappeared at the end of one week. Following the second and third injections of live bacilli there promptly appeared a raised, tender mass beneath the

[1] *Jour. Exper. Med.*, 1911, 14, p. 100.

skin which rapidly increased in size and spontaneously ruptured on the fifth day. The contents consisted chiefly of pus cells, many of which contained the acid-fast bacilli. Moreover, the injections produced a leukocytosis of 10–20 thousand.

It is thus seen that under certain conditions the introduction of leprosy bacilli into the sensitized animal is characterized not only by the accumulation of polymorphonuclear leukocytes to the point of inoculation, but also an increase of these cells in the general circulation. It is also noted that the pus cells are capable of taking up the bacilli. The factors determining the accumulation of pus cells appear to be a condition of hypersensitiveness of the animal to the bacillus as a result of which the bacilli stimulate the activity of these cells.

The results of animal experiments lead us to think that if it is possible to increase the sensitiveness of the leper to the protein constituent of the leprosy bacillus which will result in a marked tissue reaction, an activity on the part of the polymorphonuclear leukocytes may be stimulated which will bring about the destruction of the bacilli throughout the human organism. That it is possible to promote a marked alteration in the reaction of the human tissues to the bacilli the following cases prove.

In New Orleans and at the Louisiana Lepers' Home several human cases of leprosy were selected for experimental treatment with killed cultures or with the protein extract of *B. leprae.* The cases for the most part were of the mixed type and represented active phases of the disease. Some of the incipient cases were of the maculo-anesthetic type, while others presented distinct tubercles and nerve lesions. Four of the patients, previous to the initiation of bacillary inoculations, had been under routine treatment with strychnine or Chaulmogra oil or Aesenic for varying periods with negative results.

Though the treatment of human cases with bacterio-protein is still in the experimental stage and no positive statement regarding a permanent cure can be made at this time, we are justified in reporting on the progress thus far reached with its use. Suffice it to say that the proper administration of large doses of the protein constituent of *B. leprae* in early cases of human leprosy produces

a distinct and marked improvement in the patient's condition and in certain cases causes a complete disappearance of all signs and symptoms of the disease. Whether in these apparently cured cases the results are permanent time only can determine, as not infrequently there is a disappearance of all external lesions for months in the natural course of the disease.

Whitmore and Clegg[1] report negative results from the treatment of 32 cases of human leprosy in which they used a glycerin extract and soap solution of killed leprosy cultures. These authors conclude that no benefit was noticeable in any of their cases though the patients were under treatment for a period of twelve and one-half months. Since our results with the bacillary products in the treatment of leprosy do not accord with theirs, we believe the difference is explained by the fact that their dosage was too small and its administration discontinued when a severe constitutional reaction followed the injections. In our experience a constitutional reaction is highly essential and to be desired. The more severe the reaction induced by the injection, the more marked is the subsequent improvement in the patient's condition.

Case 1. A. F., 48 years of age, a man of large frame and comparatively stout. The diagnosis of leprosy was made one year before the institution of treatment although he stated that for four or five years previous he had noticed blotches appearing in his skin. During the past year he had worked only occasionally, his working time having been irregular during the latter months. He gave as the explanation a general weakness more particularly of the left hand which interfered with his grasping objects firmly. In December, 1910, he came under our observation, at this time unable to work and presenting very extensive lesions of leprosy. Practically his whole body was covered with large, firm, irregular, slightly raised, somewhat edematous, copper-colored patches. In several places, more especially over the forearm and back, these patches were from 0.5 to 2.5 cm. in the longest diameter.

The left ulnar nerve was palpable, there was almost complete analgesia and loss of power over the area of its distribution. The right hand was also numb and weak. Leprosy bacilli were found in great numbers in material from the tubercles and macules, none, however, were found in secretion from the nose. Leukocytes numbered 8,400, polymorphonuclear leukocytes, 67 per cent. A positive Wassermann and leprosy binding reaction was obtained. No agglutinins were found in dilution of 1 to 20.

Treatment by means of subcutaneous injections of bacilli killed by tricresol was instituted, December 20, 1910. The first injection consisted of four million bacilli. Treatment has been continued weekly from this time up to the present. Doses have

[1] *Philippine Jour. Sci.*, 1910, 5, p. 559.

been increased rapidly so that by March the patient was getting 2,000 million organisms at a time and since then the dose had gradually been increased up to six billion bacilli.

For the first two months although the patient claimed to feel better no objective evidence of improvement other than an increased power in the left hand was noted. During March, however, the patient's nerve lesions improved rapidly so that, according to his statement, he returned to work with complete return of power of grip.

Very soon after large doses of bacilli (over two billion) were used, it was noted that at the point of injection the day following the inoculation there appeared a firm swelling about 2.5 cm. in diameter having a reddened center and being moderately painful.

Simultaneously with the appearance of the local reactions at the site of injection it was noted that following each dose there developed in a larger or smaller number of lesions, especially those of the tubercular type situated upon the arms, an acute inflammatory reaction characterized by swelling, redness, edema, and the aggregation of polymorphonuclear leukocytes as shown by incision. These reactions appeared constantly after their beginning, for the most part different areas being involved each week, and in general the more advanced lesions showing a greater tendency to react. The first appearance of redness was evident about 18 to 24 hours after the injection. The manifestation continued to increase to the third or fourth day, gradually receding so that by the end of the week practically all edema had disappeared, and the lesions were characterized merely by a slight swelling and moderate redness. By the end of the third week after such an exacerbation not only was all evidence of the acute condition past but no leprous lesion was evident, a soft, more or less pigmented area alone remaining. In many of these "healed" areas, moreover, it was impossible to demonstrate the presence of bacilli.

As a rule, the patient complained of fever and malaise for two or three days following each injection. Upon several occasions when he was asked to return for observation his temperatures varied from 99.4° to 100.4°, 18 hours after the dose was given.

Coincidentally with the rise in temperature and reddening of the lesion a leukocytosis of from 10 to 14 thousand has been noted with a differential count of from 79 per cent to 76 per cent polymorphonuclear leukocytes. Recently the patient's blood has contained never less than 10,000 white cells, this number being found several days after inoculation. Following each injection the count rises to about 13,000 at the end of 24 hours, and to 14,000 or 15,000, 48 hours after the administration of the vaccine. From this time the number gradually falls, reaching 10,000 by the seventh day. This increase is found by differential count to consist chiefly of polymorphonuclear neutrophilic leukocytes.

At present the patient presents a definite and marked case of leprosy, but the distribution and evident activity of the lesions, especially the tubercular forms, have markedly diminished and all evidence of nerve involvement has disappeared. The lesions all over the body are less firm and the skin is softer and more pliable. The patient claims, too, to feel much stronger generally, excepting on those days when he suffers from fever.

Results of treatment: marked improvement and disappearance of all active lesions.

CASE 2. L. P., Spaniard, aged 36. A case of incipient leprosy, probably of four months' standing. Patient exceedingly well developed and nourished; fisherman by

occupation. Born in Louisiana. Presented himself at the Touro Infirmary, February 12, 1911, complaining of numbness of both lower extremities and more or less severe pain (rheumatoid in character) in the arms and legs. His chief trouble was the inability to use his left foot. He also complained of "feeling bad" and unfit for work.

On examination it was found that he had complete foot drop (left) and in places analgesic areas in the outer side of the left leg from the knee to the toes which corresponded very closely to the distribution of the peroneal nerve. There was a trophic ulcer approximately one centimeter in diameter on the outer side of the left toe. On the left buttock just above the gluteal fold there was a sharply defined elevated purplish red area measuring four centimeters in diameter. Deep incision of this area caused no pain. The normal skin immediately around this macule was hypersensitive. Smears prepared and stained from the bloody serum obtained by incising the macule showed innumerable acid-fast bacilli which corresponded morphologically and culturally to *B. lepra*. The bacilli occurred for the most part in dense colony masses within mononucleated cells. No polymorphonuclear leukocytes were demonstrable in the smears prepared from the serum free from blood.

A careful examination of the patient showed no other macular skin lesion except on the face, which was swollen and of a purplish red color, most marked over the malar prominences. The patient complained that in shaving he could not feel the razor and, as he expressed it, his face felt dead.

Examination of the nasal secretions was negative with respect to acid-fast organisms. The blood at this time gave a slight positive reaction (complement binding in the presence of leprosy culture antigen and Wassermann antigen [Noguchi]). It is noteworthy that the semen which was collected at this time by Dr. Harris not only showed acid-fast organisms in the stained preparations but yielded a culture which was subsequently identified as the leprosy bacillus. No specific organism was found though a great number of smear preparations were examined.

On February 4, 1911, the patient was given subcutaneously into the outer side of the left arm four million killed leprosy bacilli suspended in 1 c.c. of normal salt solution. No local or constitutional reaction was occasioned by the injection. One week later a second injection of dead bacilli (four million) was given subcutaneously into the right arm; again no reaction either local or constitutional followed the injection.

At weekly intervals over a period of five months the patient has received subcutaneous inoculations of killed leprosy bacilli in gradually increasing doses until four billion were being administered at a single injection. During the first two months of treatment no local or constitutional symptoms followed the inoculations. After this period, however, reactions were obtained which at first were slight. The reaction at first manifested itself as a mild erythematous patch surrounding the inoculation site, which would persist for two or three days and then disappear. Subsequent injections have given rise to hard, tender inflammatory masses which gradually increase in size and in a week or ten days fluctuate and sometimes rupture, discharging a bloody purulent material. The ulcer after rupture would heal kindly and promptly. Cultures prepared from the fluctuating tumor have in every instance proven sterile. Microscopic examination shows the material to be made up almost entirely of polymorphonuclear leukocytes, many of which are filled with acid-fast bacilli.

At this time the patient's face would appear more rough and swollen and the

macule on the buttock became more elevated and darker red in color. The smears prepared from the serum from this macule now showed great numbers of polymorphonuclear leukocytes and relatively the same number of bacilli as when first examined. From this time on, the skin lesions (face, buttock, and toe) began to subside and in two or four months after the first inoculation had completely disappeared.

After the reaction was obtained it was thought advisable to increase the interval of inoculation to two weeks and cut down the dose. Marked improvement has been noted and felt by the patient from the time the first skin reaction occurred. Gradually the rheumatoid pains disappeared and the patient in four months after the first injection recovered completely the use of his foot. The swollen, purplish color of the face has entirely subsided; the trophic ulcer on the outside of the left little toe also has healed.

The man says that he now feels as well as he ever did in his life and that he can feel the razor when shaving and "life has returned" in his limbs and arms. Clinically and bacteriologically the patient is entirely well. There are no lesions on his body and all signs and symptoms have disappeared.

It is noteworthy that at no time after the treatment was begun did the patient develop any new skin lesions and those present began steadily to retrogress after the first local skin reaction was obtained.

During the course of the treatment repeated examinations of the serum from the incised macule on the buttock were made in order to note the character of the cells and the number and appearance of the bacilli. As the lesion subsided the bacilli became fewer and fewer until at present they are no longer demonstrable. The blood has shown a moderate degree of leukocytosis (11,000 to 13,000—polymorphonuclear leukocytes 70 to 80 per cent) which is most marked in 24 to 48 hours after the injection.

Result of treatment: Apparently cured.

Case 3. White girl, aged ten years, a native of Louisiana, was admitted to the Home two years ago, having had leprosy for two or three years before admission. On admission to the Home patient presented a characteristic macular eruption bilateral on trunk and limbs and perforating ulcer on one foot. At this time the face was free from eruption, and there was no evidence of distinct tubercles on any part of the skin. Analgesia was well marked in both the upper and lower extremities, one of the lower limbs presenting a scar which was the result of an injury caused by the gnawing of a rat during sleep. Both the ulnar nerves were enlarged. Since admission to the Home the perforating ulcer has healed, but the other symptoms have persisted, and in addition a crop of tubercles has appeared on the face and arms. These nodules at the commencement of the vaccine treatment were about the size of a split pea, unusually non-inflammatory in character, varying in color from a very light brown to a darker shade. They were rounded in outline, distinctly demarkated from the intervening normal skin, cheeks, chin, and ears. Those on the forehead and cheeks were discrete, but on the chin and in the lobes of the ears they were massed together and showed evidences of continuing enlargement. There were about 50 such nodules on the face, and on the forearms about an equal number, distributed on both flexor and extensor surfaces. There was no general infiltration of the skin of the face, and the case was selected for treatment with vaccine because the individual, discrete nodules offered a good opportunity to observe possible effects of treatment, and because from the point of view of its progressions, the disease as affecting the skin was still in its incipiency.

This case was unable to take Chaulmogra oil and was progressing unfavorably under strychnine and Fowler's solution, which was discontinued when the vaccine treatment was commenced.

The first injection of vaccine was made on January 28. The dose was 0.15 c.c. of a vaccine estimated to contain 250 million bacilli per cubic centimeter. One injection a week was given, increasing to 1 c.c of a vaccine containing 800 million bacilli to the cubic centimeter. Two subsequent injections of an equal dose were given at intervals of one week. The vaccine was then temporarily stopped on account of the violence of the reaction both locally and generally. With the first small doses no reaction was observed either local or general, but as the dose was increased, the site of each injection showed the evidences of inflammation which increased in severity until large, hard masses the size of an egg formed at each puncture. These developed into abscesses after running an indolent course of a month or two. The abscesses were allowed to rupture and discharge spontaneously except one which was opened for microscopical examination of the pus contents. The discharge continued for several months and at the present writing (June 25, 1911) the most recent are still unhealed.

No constitutional reaction was observed in this case until several weeks after the last injection. Fever then developed and a curious swelling of the hands and feet. The elevation of temperature ran an irregular course of about one month's duration, the rises and falls resembling septicemia, varying from almost normal to 104° F. The swelling of the hands and feet occurred at the same time. Both hands and feet were enlarged to about one-third more than their natural size. The swelling was non-inflammatory in character, and looked like ordinary edema, but was distinctly hard to the touch, and did not pit on pressure. During the last month this swelling has gradually disappeared until at the present writing the hands and feet have regained their normal size.

Effect of treatment: At the present writing the skin of the face and arms shows a marked improvement. The only distinct nodules are on the chin and ears, where the eruption was most marked before the vaccination. These are now reduced to about half their size and number. The tubercles on the cheeks have almost entirely disappeared, leaving a brownish pigment on their former site. A similar resolution seems to have taken place in the nodules on the arms. No change has been observed in the macular eruption on the trunk and limbs.

CASE 4. White woman, aged 19 years, a native of Louisiana, admitted to the Lepers' Home three years ago, has had leprosy for eight years. Type, mixed. Stage, incipient. The condition of the patient in spite of the duration of the disease was good at the commencement of the vaccine treatment; there was no leonine expression and no claw hand. There was a macular eruption on the trunk and limbs and one slightly infiltrated macule on the cheek, also analgesia of the hand and forearm. There were no tubercles. Treatment was commenced January 28, 1911 with subcutaneous injection of seven minims of vaccine containing 250 million bacilli per cubic centimeter. Weekly injections were continued increasing the dose until March 11, 1911, when thirty minims of vaccine of 800 million per cubic centimeter were given. The local reaction was identical in character with Case 3, but was observed to come on sooner with the larger initial dose. This case also showed a similar swelling of the hands. The constitutional reaction was interesting on account of the severity of the inflammation in the old macules, which occurred during the week of the last injection. The patient was confined to bed for three weeks with temperature ranging from 102 to 103

and intense inflammation in the old lesions on the arms and legs. The legs from the knees upward were involved with an eruption which looked like erysipelas, it was elevated, red, painful; there was also pain in the joints. Exfoliation of the epidermis occurred after the subsidence of the inflammatory symptoms.

Effect of treatment: Macular eruption unchanged on trunk and limbs. Improvement in infiltrated patch in cheek.

Case 5. White woman, aged 22, a native of Texas, an inmate of the Home for three years, has had leprosy for 11 years. Type, mixed. Stage, advanced. At commencement of vaccine treatment, patient presented the characteristic appearance of leprosy of the advanced skin type, approachnig the terminal stage of the disease. The nerve symptoms were less marked. Besides the general infiltrated macular eruption and leonine facies, the respiration was difficult and speech was possible only in a whisper. The vaccine was given exactly as in other cases. The local reaction was the same as Case 4. One abscess from injection of 600 million bacilli, on February 25, 1911, remains still unhealed.

Case	Treatment	Date of Inoculation	Leukocyte count	
			Before Injection	24 to 48 Hours after Injection
Pablo	8 million killed bacilli injected subcutaneously	July 12, 11	5,600	11,000
Pablo	1 billion killed bacilli injected subcutaneously	July 20, 11	5,790	14,000
Pablo	4 billion killed bacilli injected subcutaneously	August 4, 11	6,000	21,000
Chevalier	4 billion killed bacilli injected subcutaneously	July 1, 11	7,420	12,000
Chevalier	4 billion killed bacilli injected subcutaneously	July 12, 11	8,000	15,000
Chevalier	4 billion killed bacilli injected subcutaneously	July 18, 11	5,000	21,000
Figaro	4 hundred million killed bacilli injected subcutaneously	July 12, 11	8,200	16,000
Figaro	4 billion killed bacilli injected subcutaneously	July 18, 11	5,000	21,000
Figaro	4 billion killed bacilli injected subcutaneously	July 25, 11	6,820	17,000
Smith	1 c.c. protein extract of *B. leprae*	August 14, 11	6,000	15,720
Smith	1 c.c. protein extract of *B. leprae*	August 23, 11	6,280	22,000
Smith	1 c.c. protein extract of *B. leprae*	August 29, 11	7,200	19,300

Four other abscesses have left ulcers at seat of injection which are healing nicely. Patient was confined to bed for one week after last vaccination, with fever and inflammatory symptoms in old tubercles and infiltrated macules. A few tubercles became so violently inflamed that suppuration occurred and small abscesses formed which discharged and healed.

Result of treatment: Up to date of writing, marked improvement has been noted in patient's condition.

CASE 6. White male, aged 18, a native of Louisiana, has had leprosy for 13 years and has been an inmate of the Leper's Home two years. Type of the disease is mixed, with a preponderance of the nerve symptoms. The ulnar nerve is enlarged to about the size of a man's little finger. His face and ears are covered with tubercles and infiltrated patches. The length of time that elapsed after the last injection, before any constitutional symptoms appeared, was three weeks. The temperature rose to 104° F. and the inflammatory evidences in old tubercles was characterized by pain. Marked improvement has been noted in patient's condition as a result of treatment up to present date.

DISCUSSION AND SUMMARY.

If our explanation of the factors underlying infectibility of animals by the leprosy bacillus is correct, we must assume that the epithelioid cell is more or less essential to the growth of the bacterium. Among human cases, however, it is evident that, even though these cells may be necessary in order that the bacilli can proliferate, contrary to our experience with animals, the microorganism appears able under ordinary conditions to prolong indefinitely its vitality in the human tissues. Such being the case, there remain but two methods by which we may hope to eradicate the disease; either the bacilli situated focally must be walled off, as frequently occurs in tubercle infection, or the host conditions must be so altered that the bacilli will be unable to live. The former process is at best unsatisfactory and appears almost impossible of procuring, as leprous lesions disclose little tendency toward fibrous tissue encapsulation. There remains, therefore, but the possibility of altering conditions, which theoretically can be procured either by the development of substances in the body fluids inimicable to the growth of the organism, that is, the commonly appreciated immune bodies, or the stimulation of cells which are capable of destroying the bacilli by phagocytosis. Since the production of the development of immune bodies is comparatively inadequate it would seem that only by phagocytosis can the bacilli be destroyed.

It is apparent that the epithelioid type of cells (including the lymphoid and plasma cell) is comparatively useless as a destructive agent, if not positively helpful to the growth of the bacterium. On the other hand, there is every proof that the polymorphonuclear leukocyte is capable of destroying the leprosy bacillus in a manner similar to that in which it destroys the pyogenic cocci, etc. We

find that although such cells are usually present in small numbers in human leprous lesions, only infrequently are many found.

Since both the leprosy bacillus and the disease of leprosy have many points in common with the tubercle bacillus and tuberculosis respectively, it is justifiable to discuss in this paper phenomena noted in the latter disease. Our experience suggests, moreover, that certain conditions which are of interest and importance in the study of tuberculosis are exemplified by observations upon experimental leprosy, more particularly, the reactions of a focal character and the subsequent changes in the lesions which follow the inoculation of dead bacilli or the bacterio-protein.

The bacillary products of *B. leprae* like those of *B. tuberculosis* are relatively non-toxic in the ordinary sense of this term; they will not readily poison a normal animal (enormous doses in relation to body weight being necessary) nor will they stimulate antibodies in the host in amounts directly proportionate to the quantity of "leprosin" introduced.

In view of the difficulty experienced in the production of a protective immunity against the tubercle bacillus and the comparative absence of agglutinins, opsonins, amboceptors, etc., in the serum of animals and patients recovering from or cured of an infection by the tubercle bacillus, numerous theories have been brought forward to explain the cure of this disease.

Baldwin[1] believes that in the employment of tuberculin in the treatment of tuberculosis two factors are important, namely, immune-body production and reactive inflammation at the site of the individual lesions. This author makes, however, no attempt to explain why the focal reaction takes place nor the manner in which it influences favorably the subsequent course of the disease.

Recently[2] Krause in an article based upon experimental as well as human studies of immunity to tuberculosis states as his opinion that the ordinary tuberculin reaction in animals and in patients is, perhaps, due to the absorption of toxic material from the lesions irritated as the result of injection rather than due to the toxicity of the material inoculated. Further, he writes: "Since we know that tuberculo-protein inflames the tubercle it is more than likely that the end result of every dose, no matter how slight, is to bring about changes that may vary from the most transient hyperemia to the most intense inflammation of the focus. The purpose of such changes would be, of course, conservative and their end would be fibrosis." He likens tuberculin treatment to the employment of silver nitrate, copper sulphate, etc., in the treatment of chronic ulcers.

[1] *Jour. Med. Res.*, 1910, 22, p. 189.

[2] *Ibid.*, 1911, 24, p. 3.

Krause's observations we believe represent the most modern and apparently the most correct view relative to the method of action of tuberculin in the therapy of tuberculosis. Our experience in dealing with the products of *B. leprae* is in accord with his regarding the function of tuberculin. Since, however, we have in leprosy a disease which is characterized by less danger from toxic reactions, it is justifiable to employ more heroic measures in the treatment of this disease than in tuberculosis. Furthermore, leprosy offers a better opportunity than tuberculosis for the study of the immunity principles underlying both infections. In order, however, to explain the phenomena occurring in the process of infection and cure of such diseases as tuberculosis and leprosy, it appears that some theory more elaborate than those commonly considered must be conceived.

The following appears to us to present a working hypothesis concerning the changes taking place within the body following the administration of a dose of leprous protein. As stated above, it appears that the growth of the bacilli continues normally because the organisms themselves are of the nature of non-irritating foreign bodies which proliferate within cells of the epithelioid type. It is probable that there constantly takes place a splitting of certain numbers of bacilli with the freeing of toxic products; usually, however, the anti-anaphylactic bodies directed against these toxic substances are present in sufficient quantity to render them at once inocuous, in other words, the absence of cellular (polymorphonuclear) and febrile reaction is due to a balance having been established between the bodies. By means of the subcutaneous injection of leprous protein a comparatively large amount of toxic body is liberated as the material is at once brought into contact with the body fluids. As a result of this increase in toxic products there is a withdrawal to the spot of the available antitoxic body. When this occurs any splitting of leprosy bacilli situated focally will result in the bacilli in these areas acting as toxic bodies of sufficient potency to determine the accumulation of polymorphonuclear leukocytes following perhaps their liberation from the epithelioid cells as the result of the destruction of the latter by the toxin. Thus would be brought to bear upon the bacilli whatever immune bodies were present in the body fluids owing to the loss on the part of the

bacilli of the protection of the epithelioid cells as well as the destructive properties of the pus cells.

It is comparatively easy to understand why, in an individual in whom there is a balance of split protein and antisplit protein bodies, the introduction of material in such a manner that it is liable to rapid splitting into toxic molecules should result at the point of inoculation in a constitutional disturbance and in a local reaction. Why, however, such an inoculation should result in focal reactions at the sites of infection is much less readily understood. For the present we can see no hypothesis which explains this phenomenon other than the disruption of the balance between toxic split protein and the antibody of such material. If we consider that as a result of this lack of balance each individual lesion becomes a toxic focus we can conceive of the factors determining the occurrence of hyperemia and the presence of leukocytes.

It is well known that sterile soluble or insoluble material, such as catgut, silk, or many of the metals imbedded in the tissues, do not attract pus cells. If, however, the catgut or silk be saturated with turpentine or croton oil an abscess develops. In a similar manner it seems that leprosy bacilli ordinarily act upon the tissue much as sterile catgut, etc., but that under certain conditions they act as irritants of sufficient activity to lead to the accumulation of pus cells. Experimental evidence proves that the acquired irritability is of the nature of an anaphylactic or allergic toxicity acting in a matter similar to that just described.

The chronicity of leprosy, which is one of the most marked characteristics of the disease, might be accounted for by the fact that the protein content of the casual agent is not readily split and in consequence there is apparently no reaction on the part of the host which is sufficiently potent to cause destruction of the bacilli. As a result of this lack of toxicity the reactive forces of a useful type are not developed.

This view is similar in many respects to von Pirquet's explanation of the apparent immunity of individuals previously infected by diseases such as vaccinia, smallpox, etc., namely, that the freedom of such persons from subsequent infection is not based upon an acquired insensibility against the virus causing the disease, but to

an early reaction taking place upon the introduction of the causative agent.

Such an explanation of the apparent immunity of individuals who have recovered from infection by one or other of the exanthemata is satisfactory to a certain degree, but explains insufficiently the focal reactions which occur in both tuberculosis and leprosy. We believe that the local allergic reaction of von Pirquet and the focal reactions at the site of the lesions can be better explained by considering that as the result of a hypersensitive or anaphylactic state the virus or protein substance which, to the normal person, is non-toxic and therefore causes no local irritation and hence excites no reaction is at once split into a toxic substance by the previously manufactured toxic bodies. As a result of the rapid freeing of toxines a reaction of greater or less intensity at once ensues, resulting in the destruction of the invading virus. According to our conception, the reaction is the result, not of the death of the organism through its binding with the antibody, but of the split protein-toxic body, and the reaction by means of the cells involved brings about the death of the virus.

The phenomenon of acute exacerbation of the leprous lesions occurring from time to time during the course of the disease accompanied by fever is well known. It has also been noted by clinical observers that following attacks of fever the lesions which have been involved in the more active process have a tendency to disappear. It has been pointed out, moreover, that the increased redness, swelling, and pain in the lesions are accompanied by all the microscopic evidences of an acute inflammatory reaction, including large numbers of polymorphonuclear leukocytes. Therefore the reactions obtained in the human leper by the injection of dead bacilli are analogous to those which develop naturally in certain cases (so-called lepra fever). Our results justify also the belief that the same satisfactory end result is obtained, namely, improvement in the lesions involved in the acute reaction. Since we are in a position to control by inoculations the severity and extent of the reactions both local and constitutional, it appears that we have at our disposal a means of permanently and markedly ameliorating the condition of the leper.

In the treatment of leprosy with killed cultures of the bacilli,

whether the whole bacilli or the extracted proteins are used it would seem essential to give large doses and often repeated, at least until the local anaphylactic reaction appears. The dose should be not less than 400 million and should be increased gradually at weekly intervals until a marked local reaction is obtained, at which time the number of killed bacilli administered is three to eight billions, or the equivalent of this number if the protein is used. In some cases the local reaction occurs after the third or fourth injection; in others not until 15 or 20 injections have been given. Once this reaction develops, the dose may be lessened and the interval between administration increased. We have followed the plan of administering subsequent injections as soon as the local reaction and leukocytosis following the previous injection have subsided.

A careful study of the blood changes during the course of treatment is important, as the rise and fall of the polymorphonuclear leukocytes is a most excellent control of the dose to be given and the intervals to observe between injections. We have found that there is induced a marked leukocytosis after each injection which diminishes *pari passu* with the subsidence of the local reaction, the period of recrudescence varying from three days to two weeks, depending on conditions. Therefore, at the time of subsidence of the leukocytosis (see table), another dose should be given if the best results are to be obtained.

Whereas, in the treatment of tuberculosis anything but minute doses is liable to do serious harm, large doses of the specific bacterio-protein in the treatment of leprosy can be employed with impunity. In fact, leprosin must be given in large doses and often repeated if beneficial results are to accrue. In other words, leprosin, unlike tuberculin, can be pushed to the limit.

In none of the cases treated by us have new lesions developed after the local reaction was obtained. In early cases there is a complete subsidence of all visible lesions and other signs of the disease in four to six months after systematic treatment has been begun and carried out. We believe, therefore, that the proper administration of the product of *B. leprae*, whether the protein extract or the whole killed bacilli are used, will not only ameliorate the condition of the leper but in early cases, including both types of the disease, will bring about a permanent cure.

SPECIFIC ANTIBODIES IN SCARLET FEVER.*†

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The demonstration of specific antibodies in scarlet fever has been limited chiefly to those for streptococci. The numerous investigations which deal with this subject, carried out with the object of determining the rôle which the streptococcus plays in the etiology of scarlet fever, may here be reviewed in brief, before we proceed with the report of our own work.

Antibodies for Streptococci.

I. *Agglutinins.*—Grünbaum[1] seems to have been the first to report on the agglutination of streptococci of scarlet fever by the serums of scarlet-fever patients. He simply states that he was able to isolate a coccus from a case of scarlet fever which agglutinated with scarlet-fever serum.

Baginsky and Sommerfeld[2] examined the serums of scarlet-fever patients in the third to the sixth week of the disease, for specific agglutinins of streptococci, which were cultivated from cases of scarlet fever. Their results were absolutely negative. At about the same time Moser and von Pirquet[3] found that in 52 per cent of the cases examined the serum caused marked agglutination of scarlatinal streptococci. The serum from cases other than scarlet fever gave the reaction in a much smaller percentage and only on higher dilution.

Salge and Hasenknopf[4] made a comprehensive study of this question. They found that emulsions of streptococci of scarlet fever were markedly agglutinated by the serum of scarlatina in a dilution as high as 1:500, whereas the reaction failed with streptococci from other sources, even with a lesser dilution of the serum. This specific property of the serum vanishes toward the end of convalescence.

Dopter[5] denies the specific relation of scarlet-fever serum toward homologous streptococci. In his hands streptococci of various origin were agglutinated by the serum of scarlet-fever patients and indeed in some cases the serum did not react with streptococci from scarlet-fever cases. Dopter reaches the conclusion that the streptococcus is not the specific agent of the disease.

Detot,[6] and Detot and Boucart[7] arrived at similar results. Since the agglutination is not specific they reject its application as a diagnostic means and deny the rôle of the streptococcus in the etiology of scarlet fever.

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[1] *Science Progress*, 1897, 1, p. 616; *Lancet*, 1897, 1, p. 451.

[2] *Arch. f. Kinderh.*, 1902, 33, p. 1.

[3] *Centralbl. f. Bact.* I, Orig., 1904, 36, p. 692.

[4] *Jahrb. f. Kinderh.*, 1903, 58, p. 218.

[5] *Compt. rend. Soc. de biol.*, 1904, 56, p. 787.

[6] *Ibid.*, 57, p. 44.

[7] *Rev. mens. de malad. de l'enfance*, 1905, 23, p. 64.

In the same year there appeared an important article by Weaver[1] on the agglutination of streptococci by scarlet-fever serum. He too comes to the conclusion that the agglutinins in the serum are not specific; that in the serum of patients with erysipelas, pneumonia, and even other infections of non-streptogenous nature, agglutinins for scarlet-fever streptococci may be present. Weaver points out further that the discordant results of the different investigators may be due to the varying composition and reaction of the culture medium.

Jogiches[2] found that the serum of scarlatina agglutinated streptococci of the most different origin even in dilution of 1:500 and 1:600; the reaction appears in about the fifth to the sixth week of the disease, but fails entirely in the beginning of the disease. There exists no constant difference in the intensity of the reaction toward the various strains of streptococci.

Rossiwal and Schick[3] found several streptococci, biologically different, in one case of scarlet fever, which gave various high agglutination figures with Moser's antistreptococcus serum.

Rüdiger,[4] on the other hand, found that the serum of a sheep immunized against a streptococcus isolated from the throat of scarlet-fever cases contained specific agglutinins for this strain only, and that the *Streptococcus viridans* was not agglutinated.

II. *Bacteriolysins* —Weaver and Rüdiger[5] have shown that neither normal nor scarlet-fever serum contains lysins for streptococci.

III. *Phagocytosis and Opsonins.*—The importance of phagocytosis in streptococcus infections was demonstrated by Metchnikoff in the case of erysipelas. Denys and Leclef[6] showed that immune serum promotes the phagocytosis of streptococci, though the term opsonin was not then used. The important contributions of Denys and Marchand, Bordet, Hektoen, Neufeld and Rimpau, and Rüdiger cannot be considered here, as they deal mostly with the mechanism of experimental streptococcus infections, while we limit ourselves here to the production of antibodies and their demonstration in scarlet fever.

Ruth Tunnicliff[7] found that in the beginning of the disease the streptococco-opsonic index is below normal in the majority of cases. As the acute symptoms subside, the index rises above normal, to which it returns, at times, quite abruptly; and in uncomplicated cases it usually remains normal during convalescence. Definite localized streptococcus complications are inaugurated by a depression in the streptococco-opsonic index, which rises again as improvement sets in. These variations of the opsonic index are limited to the streptococcus and are absent with respect to pneumococcus, staphylococcus, and pseudodiphtheria bacillus; hence "the streptococco-opsonin in scarlet fever is a specific opsonin."

Banks[8] arrives at similar results: In cases of scarlet fever running a normal course the opsonic power is decreased during the early febrile period and rises to normal or above normal with the defervescence. In fatal cases the opsonic power is markedly subnormal. Complications alter the curve; the opsonic power is decreased at the onset and during the earlier period of albuminuria, severe nephritis, and secondary adenitis. Banks believes in a definite relationship of the *Streptococcus scarlatinae* to the disease, yet there is no striking difference in the results obtained with different varieties

[1] *Jour. Infect. Dis.*, 1904, 1, p. 91.

[2] *Centralbl. f. Bact.*, I, Orig., 1904, 36, p. 692.

[3] *Wien. klin. Wchnschr.*, 1905, 18, p. 3.

[4] *Jour. Infect. Dis.*, 1906, 3, p. 755.

[5] *Trans. Chic. Path. Soc.*, 1903, 5, p. 285.

[6] *La cellule*, 1895, 11, p. 175.

[7] *Jour. Infect. Dis.*, 1907, 3, p. 304.

[8] *Jour. of Path. and Bact.*, 1907, 12, p. 113.

of streptococci. These findings of Tunnicliff and of Banks were confirmed later by Jochmann and Michaelis.[1]

IV. *Antitoxins.*—All investigators, who have occupied themselves with the proof of antitoxic substances in the serum of scarlatinal patients, have arrived at negative results. Neither Besredka, nor Neufeld, nor Moser and Schwoner were able to demonstrate antistreptocolysins (antihemotoxins) in the serum.

V. *Bordet's Antibodies for Streptococci.*—Besredka and Dopter[2] examined the serum of scarlatinal patients for complement-deviating antibodies for streptococci. Besredka himself previously had found with this method antibodies in streptococcus-immune serums. As antigen they used streptococci cultivated from the throat and blood of scarlet-fever patients. As they were not able to find a specific "fixateur" in a single case they considered themselves justified in concluding that the streptococcus plays no specific part in scarlet fever, but has the rôle of a secondary participant. Foix and Mallein[3] repeated these experiments with more positive results. They used a polyvalent antigen of streptococci which had been cultivated from numerous cases of scarlatinal angina. Though the cases examined are small in number the results obtained are interesting. With twelve serums of scarlet-fever cases they obtained deviation of the complement ten times. Controls made with serum of other streptococcus infections, as erysipelas and puerperal sepsis showed complete hemolysis. The antibodies were present as early as the fourth, and as late as the 38th, day.

Livierato[4] examined 18 scarlatinal serums using as antigen extracts of streptococci, staphylococci, and pneumococci, typhus, colon, and influenza bacilli. Complete inhibition of hemolysis was obtained by the streptococcus extract only, and this in every case, though the streptococci used were not cultivated from cases of scarlet fever. Livierato considers these findings an important contribution toward the proof of the streptocogenous nature of scarlet fever.

H. E. Eggers[5] obtained "rather rough positive reactions in a certain proportion of cases, but could not obtain the reaction in all cases." He points out the important fact that broth cultures of streptococci used as antigen may be a source of error in performing the reaction, as sterile broth in itself possesses complement-fixing (antihemolytic?) properties.

Schleissner[6] used suspensions of streptococci from scarlatina, erysipelas, puerperal sepsis, and panophthalmia as antigen. The serum of scarlatina, especially from the seventh to the 35th day, reacted positively with scarlatinal streptococci, whereas control serums from other affections never gave a positive reaction. With erysipelas streptococci, scarlet-fever serums never gave deviation, though some reacted positively with the streptococci of puerperal sepsis and panophthalmia. This latter reaction, however, was quantitatively weaker than the reaction with the scarlatinal streptococci.

From all these investigations it results clearly that the serum of scarlatina patients contains antibodies for the streptococcus. This speaks undeniably for the biological relation of this microorganism to scarlet fever. The existence of a specific scarlatinal streptococcus, however, is not demonstrated by this work, and no light has been shed on the primary etiology of the disease. The very presence of antibodies cannot solve the problem whether the streptococcus is the primary agent of the disease or a secon-

[1] *Berl. klin. Wchnschr.*, 1910, 47, p. 921.
[2] *Ann. de l'Inst. Pasteur*, 1904, 18, p. 373.
[3] *Presse méd.*, 1907, 15, p. 777.
[4] *Centralbl. f. Bact.*, I, 54, p. 422.
[5] *Chic. Path. Soc.*, 1908, 7, p. 166.
[6] *Folia serologica*, 1909, 3, p. 271.

dary invader. And even if it had been shown that these reactions were obtainable only with scarlatinal serum, and streptococci cultivated from scarlatina, the question of the etiology of the disease would still be unsolved. In this connection the work of DeWael and Sugg[1] may be recalled. They found that the streptococci, constantly present in smallpox, are specifically and exclusively agglutinated by the serum and other serous liquids of variola patients and convalescents (dilution 1:400–1:800), but not by any other immune streptococcus serum. Yet one would hardly conclude from this that the streptococcus is the etiologic agent of variola.

It therefore seemed appropriate to use those methods for the examination of antibodies which have proved valuable in other diseases of unknown origin, and to exclude as far as possible the streptococcus and its antibodies in the arrangement of the experiment.

SPECIFIC ANTIBODIES IN SCARLET FEVER.

For the demonstration of antibodies in diseases, the germ of which is unknown or incapable of cultivation, two methods may be employed: one rests on the precipitating action, and the other on the complement-binding function, of the anti-serum. Complement deviation offers several advantages over precipitation; these are chiefly:

1. Deviation of the complement occurs on the use of quantities of antigen so small as not to show precipitation.

2. Complement-binding substances appear in the serum at a time when precipitins cannot be demonstrated (Muir and Martin).

3. The complement-binding antibodies are more specific than the precipitins (Bauer-Bruck).

It seemed therefore preferable to use the binding of the complement for the attempt at demonstration of specific antibodies in scarlet fever. Such attempts are not new. In the last few years numerous reports on complement fixation have appeared, instigated by the report of Much and Eichelberg that they obtained fixation of complement with scarlet serum and syphilitic antigen (positive Wassermann reaction). Almost all the following investigations were undertaken with the view only of testing the credibility and specificity of the Wassermann reaction, without consideration of the question as to the presence of specific antibodies in scarlatina.

The fact that some scarlatinal serums give, with some syphilitic extracts, complement deviation, though not impairing the practical value of the Wassermann reaction for syphilis, is of the

[1] *Arch. intern. de pharm. et de thérap.*, 1903, 12, p. 205.

greatest importance in respect to the use of Bordet's and Gengou's phenomenon as a method of searching for antibodies for an unknown etiologic agent in scarlet fever.

The explanation of a positive Wassermann reaction in some cases of scarlet fever may lie in particular properties of the extract of the serum, or in the interaction of both. Thus, for instance, it is obvious that the presence of streptococci, or their soluble substances, in the antigen may give rise to complement deviation with scarlatina serums. This holds for watery organ extracts as well as for alcoholic ones. It was shown by Rüdiger that suspensions of organ extracts have no inhibitory effect on the multiplication of streptococci. As far as alcoholic extracts are concerned, it may be emphasized that our conceptions regarding the insolubility of bacterial antigens in alcohol deserve complete revision. Thus, for instance, Levaditi and Mutermilch[1] have recently proved that those antigenetic constituents of cholera vibrios which are essential for the deviation of complement are soluble in 85 per cent alcohol. What is true for the cholera vibrios must be conceded may be the case of other microorganisms. In the selection of a proper antigen for the investigation of antibodies in scarlet fever this fact must be borne in mind.

Concerning previous work with the aim of finding whether specific antibodies are present in the serum of scarlet-fever patients, with the exception of antibodies for streptococci, the following may be mentioned:

Handel and Schultz,[2] in the second part of their paper, report complement deviation by the serum of scarlet-fever patients on the use of an extract of the liver of a child who had died of a complication of scarlet fever (empyema). With a watery extract they obtained complete complement fixation in 24 cases of 31. The authors emphasize that definite conclusions cannot be drawn from these results with regard to the complement deviation in scarlet fever with homologous extracts. The chief difficulties which arise in estimating the result of these investigators seem to be the following:

1. The antigen (watery extract of the liver) was obtained from a child who had died of an empyema, thus of a secondary infection.

2. No facts as to the etiology of this empyema are given.

Since it possibly was a case of streptococcus infection, the extracts should have been tested with serum of patients suffering from streptococci infection and with streptococcus-immune serum. The positive findings might have been called forth by streptococci or by the related germ causing the empyema.

[1] *Compt. rend. Soc. de biol.*, 1908, 64, p. 406.

[2] *Ztschr. f. Immunitätsf.*, 1908, 1, p. 91.

Uffenheimer[1] used as antigen the serum of children in the very first stages of the disease and as antibody the serum of convalescents. These experiments proved entirely negative; no inhibition of hemolysis occurred. Uffenheimer, probably rightly, considers the fault of his experiments to lie in too slight concentration of the antigen.

Sommerfeld[2] also used as antigen a watery extract of the liver of scarlet-fever victims. Of 40 serums, 18 reacted positively, 22 negatively. The paper contains no reference regarding the testing of the extract with streptococcus-immune serums.

Hecht, Lateiner, and Wilenko[3] examined 119 scarlet-fever serums for complement fixation, using alcoholic extracts of liver of scarlet-fever patients, all with negative results. They came to the conclusion that no specific properties are present in the scarlet-fever liver.

These investigations, with the exception of Uffenheimer's, are shaped directly after Wassermann's method. So far as the demonstration of Bordet antibodies is concerned, this could be regarded only as an advantage. It is, however, different if we take into consideration the hypothetical basis and the conditions for such experiments. By far the most important of the preliminary questions is the selection of an appropriate antigen.

ANTIGEN.

In the selection of an appropriate antigen it seemed to us a *conditio sine qua non* to choose such organs as seemed to speak clinically as well as anatomically for a place of predilection of the virus. These conditions seem best fulfilled by the kidney, lymph glands, and scales of the skin. We also used liver in order to control the results of previous investigations.

The conditions which an appropriate antigen must fulfil for use in the search for a specific scarlatinal virus seemed to us to be the following:

1. The organs must come from an acute, toxic case of scarlet fever in which the patient succumbed rapidly and without secondary infections and complications.

2. The blood of the patient examined in the last days before death should contain no streptococci.

3. The blood serum ought further, on being tested with an active scarlatinal streptococci emulsion, to show no deviation of the complement. The activity of the streptococcus antigen used should be tested with the corresponding immune serum.

[1] *Münch. med. Wchnschr.*, 1909, 56, p. 2471. [2] *Arch. f. Kinderh.*, 1909, 50, p. 38.

[3] *Wien. klin. Wchnschr.*, 1909, 22, p. 523; *Ztschr. f. Immunitätsf.*, 1909, 2, p. 356.

4. The watery organ extracts tested with antistreptococci-immune serum should show no deviation of complement.

From the specially mild character of the scarlet fever in Chicago, it was clear that it was not easy to find such foudroyant toxic cases as answered to these four conditions. We succeeded after long preliminary tests in finding three cases the conditions in which corresponded to all the demands which we had placed on a suitable antigen.

I. *A. S.*—Sick two days and removed to hospital on third. Three years old. No previous diseases. Maximum temperature 104.6.° Great difficulty on swallowing, slight swelling of the cervical lymph glands. Very marked, fiery-red exanthem, relative bradycardia and arrhythmia, delirium, slight albuminuria. Death on the fifth day.

II. *E. W.*—Entered hospital on the second day of the disease. Boy, nine years old. Only previous disease was measles. Maximal temperature 103.8, angina, exanthem, symptoms of cerebral irritation, tachycardia. Death on the fourth day.

III. *H. B.*—Boy, six years old. Entered hospital on the fourth day of the disease. Angina, exanthem, albuminuria, nephritis on the tenth day. Death occurred on the 15th day with uremia.

The organs were removed from the bodies a few hours after death. We used only watery extracts in our experiments, prepared in the following way: The organs were cut up into small pieces with scissors and then with the addition of physiologic salt solution containing 25 per cent phenol rubbed up into a fine emulsion and dried in the vacuum into a hard mass. This was rubbed up into a fine powder and preserved in amounts of 1 gm. in sealed tubes.

For the experiment one-gram powder for every 30 c.c. of physiologic salt solution plus 0.25 per cent phenol was taken. The mixture was shaken for two hours in the shaking apparatus and then placed in a brown bottle in the icebox. After 48 hours the extract is ready for use. The required amount is rendered clear by centrifugation immediately before use.

The kidneys, lymph glands, and liver were treated in this way.

The scales were obtained from a large number of cases. In order to macerate them completely, we allowed them to stand in a 15 per cent antiformin solution for 72 hours. The excess of alkali was removed by the addition of 5 per cent sulfuric acid, drop-wise, and of chlorine by a 5 per cent sodium-hyposulfite solution.

The streptococcus emulsion which was used as control was

prepared from blood-agar cultures of streptococci obtained by blood culture from scarlet fever, and suspended in physiological salt solution, the growth of one tube to about 2 c.c. salt solution. Before use this emulsion was tested for its inhibiting and hemolytic effect and that amount employed as a test of the serum of moribund cases, which gave complete hemolysis with the system. For instance:

Streptococcus Emulsion	Physiologic Salt Solution	Complement	Amboceptor (3 × Hemolytic Dose)	Blood	Result
1.0 c.c.	1.0 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	No hemolysis
.5 c.c.	1.5 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	Hemolysis
.4 c.c.	1.6 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	"
.3 c.c.	1.7 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	Slight hemolysis
.2 c.c.	1.8 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	" "
.15 c.c.	1.85 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	Complete "
.1 c.c.	1.9 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	" "
.05 c.c.	1.95 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	" "

The extracts were used in amounts of 15 per cent in testing the scarlet-fever serum. Only the scarlatinal streptococcus emulsions that gave complete inhibition with streptococcus-immune serums were used.

ANTISERUMS.

The serums of scarlet-fever patients were obtained in all periods of the disease, from the fourth day up to the 102d day. The blood was collected sterile by puncture of the vein of the arm. After coagulation was complete, the serum was pipetted off, centrifugated, and when entirely clear, heated at 56° for 30 minutes. We employed only inactivated serum, which was obtained on the day of the experiment.

COMPLEMENT.

Fresh guinea-pig serum was used as complement. It was titrated exactly against the hemolytic system and twice the multiple of the dose of complement was used, which just caused complete hemolysis.

HEMOLYSIN.

As amboceptor was used the serum of rabbits immunized against sheep's blood. In order to know whether normal amboceptor for sheep's blood was contained in the human serum, we tested this every time without the addition of antigen and amboceptor

(see Tube No. 4 in Example 2). If by this control test we obtained hemolysis, we removed the natural sheep amboceptor by absorption and then repeated the experiment. The procedure employed was as follows: 3 c.c. inactivated serum and 1 c.c. sheep's blood corpuscles, washed three times, were allowed to remain in contact for one-half hour in the icebox. Then they were centrifugated, and the reaction was carried on with the serum, which was pipetted off.[1]

BLOOD.

The blood was a 5 per cent suspension of sheep corpuscles in physiologic salt solution.

CONTROLS.

Each extract was tested: (1) with antistreptococcus-immune serum, (2) for hemolytic (hemotoxic) action, (3) for anticomplementary action, (4) with normal serum, and (5) with serums of other diseases, such as measles, erysipelas, nephritis, etc.

The normal extracts which served as controls were tested in the same way.

Each serum was tested: (1) for anticomplementary action (without antigen), (2) for hemolytic action without antigen and without amboceptor, and (3) with normal extract.

After titration of the amboceptor and complement and examination of the organ extracts, the chief experiment was made in the following way:

EXAMPLE OF EXPERIMENT.

Tube	Extract	Serum	Complement	Amboceptor	Blood	Result
1 ...	.2 lymph gland scarlet	.2 scarlet	1 c.c.	1 c.c.	1 c.c.	No hemolysis
2 ...	1.1 " " "	.1 "	1 c.c.	1 c.c.	1 c.c.	" "
3 ...	....	.2 "	1 c.c.	1 c.c.	1 c.c.	Complete "
4 ...	....	.2 "	1 c.c.	.....	1 c.c.	No "
5 ...	.4 lymph gland	....	1 c.c.	1 c.c.	1 c.c.	Complete "
6 ...	.2 " "	....	1 c.c.	1 c.c.	1 c.c.	" "
7 ...	.2 " "	.2 normal	1 c.c.	1 c.c.	1 c.c.	" "
8 ...	.1 " "	.1 "	1 c.c.	1 c.c.	1 c.c.	" "
9 ...	.4 normal lymph gland	....	1 c.c.	1 c.c.	1 c.c.	" "
10 ...	.2 " " "	....	1 c.c.	1 c.c.	1 c.c.	" "
11 ...	.2 " " "	.2 scarlet	1 c.c.	1 c.c.	1 c.c.	" "
12 ...	.1 " " "	.1 "	1 c.c.	1 c.c.	1 c.c.	" "
13 ...	.2 " " "	.2 normal	1 c.c.	1 c.c.	1 c.c.	" "
14 ...	.1 " " "	.1 "	1 c.c.	1 c.c.	1 c.c.	" "
15 ...	....	....	1 c.c.	1 c.c.	1 c.c.	" "
16 ...	....	....	1 c.c.	1 c.c.	1 c.c.	No "
17 ...	....	....	.....	.....	1 c.c.	" "

[1] We have used this control for more than one year in our routine work with the Wassermann reaction and can recommend it most warmly, since negative reactions with surely syphilitic serum can be avoided in this way.

TABLE 1.

Case	Name of Patient	Age in Years	Day of Sickness when Blood Was Examined	Kidney Extract	Liver Extract
1	M.B.	10	3	–	–
2	D.G.	8	6	–	–
3	H.B.	7	14	+	+
4	J.C.	9	11	+	+
5	N.L.	10	14	+	+
6	S.F.	1.5	7	–	–
7	S.L.	8	15	+	–
8	L.E.	5	16	+	–
9	W.G.	16	19	+	+
10	B.	7	8	–	–
11	L.M.	8	12	+	+
12	L.P.	12	13	+	+
13	C.G.	12	12	+	–
14	B.F.	2.5	11	–	–
15	D.A.	16	4	–	–
16	W.B.	3	7	–	–
17	W.L.	3	18	+	+
18	B.K.	24	13	+	–
19	S.E.	5	9	+	–
20	K.O.	5	13	+	+
21	D.V.	5	8	–	–
22	D.R.	10	8	–	–
23	G.R.	15	13	+	+
24	R.R.	5	19	+	+
25	W.E.	49	16	+	–
26	B.M.	3	5	–	–
27	B.A.	4	7	–	–
28	S.M.	6	19	+	+
	S.M.	6	42	+	+
29	A.H.	7	14	+	+
30	D.M.	8	5	–	–
31	J.R.	7	9	–	–
32	C.B.	3	17	+	+
33	A.M.	22	11	+	–
34	L.C.	18	16	+	–
35	V.H.	3	9	–	–
36	B.J.	5.5	13	–	–
37	C.E.	16	14	+	–
38	W.B.	17	19	+	+
39	R.G.	4	21	+	+
40	B.J.	16	5	–	–
41	K.M.	5	11	–	–
42	M.K.	5	14	+	+
43	B.M.	4	9	+	–
44	B.D.	5	10	–	+
45	L.W.	21	3	–	+
46	K.E.	15	12	+	+
47	R.R.	8	19	+	–
48	S.H.	4	16	+	+
49	C.M.	8	5	–	–
50	G.J.	12	10	–	–

In this way each scarlet serum was tested with the various organ extracts.

Beside normal serum, serums of measles, diphtheria, erysipelas, malignant tumors, tuberculosis, and nephritis were used as controls. None of these control serums prevented hemolysis with the extract.

We first examined 50 cases with liver and kidney extracts. The results are given in Table 1. Further, 25 cases with lymph glands, kidney and liver extracts (Table 2). Since the extract of lymph

TABLE 2.

Case	Name of Patient	Age	Day of Sickness when Blood Was Examined	Lymph Gland Extract I	Kidney Extract	Liver Extract
51	P.L.	20	11	+	−	−
52	H.E.	7	15	+	+	+
53	C.A.	15	9	+	−	−
54	K.E.	30	13	+	+	−
55	R.S.	8	12	+	−	−
56	S.R.	4.50	8	−	−	−
57	L.C.	25	15	+	+	+
58	P.S.	7	9	−	−	−
59	K.A.	6	17	+	+	−
60	R.R.*	$1.66\frac{2}{3}$	19	+	+	+
61	G.M.	2.50	8	−	−	−
62	B.B.	18	12	+	+	−
63	H.L.	30	7	−	−	−
64	D.M.	28	11	+	+	−
65	L.F.	12	15	+	+	+
66	L.M.B.	28	9	+	−	−
67	J.A.	24	11	+	+	+
68	B.S.	7	14	+	+	−
69	R.R.	11	13	+	+	−
70	E.H.	23	15	−	+	+
71	M.A.	24	14	+	−	−
72	W.C.	8	19	−	+	+
73	S.L.	9	11	+	−	−
74	B.G.	6	15	−	+	−
75	W.V.	4	13		−	−

* Had scarlet fever twice in one year.
+ Designates inhibition of hemolysis.
− Designates hemolysis.

glands proved the most effective, far more so than all the others, we limited our further work to experiment with this extract alone (Table 3).

The extract prepared of the scales proved negative in all instances and therefore no reference is given to this work here.

If now the positive reactions recorded in the three tables are compared, we see that:

Of 75 cases, 43 were positive with kidney extract, 57.3 per cent.
Of 75 " 25 " " " liver " 32 "
Of 118 " 81 " " " lymph-gland extract, 68.6 per cent.

Thus the extract prepared from the cervical lymph glands proved the most powerful antigen.

An extract prepared from a normal kidney of a scarlet-fever case and from one of a case of scarlet-fever nephritis showed no difference in activity.

It is striking that of the serums examined of cases of nephritis scarlet fever, all gave a positive reaction in each case.

TABLE 3.

Case	Name of Patient	Age	Day of Sickness When Blood Was Examined	Lymph Gland Extract II
76	A.R.	7	11	+
77	D.V.	6	15	+
78	F.R.	5	9	—
79	F.M.	7	12	+
	F.M.	7	58	+
80	P.M.	16	17	+
81	K.A.	10	12	+
	K.A.	19	31	+
82	W.F.	9	4	—
83	B.A.	8	17	+
84	T.N.	20	13	+
85	L.F.	9	16	+
86	M.H.	12	29	+
87	B.R.	22	14	+
	B.R.	22	46	+
88	E.A.	9	12	—
89	Z.J.	7	5	—
90	M.K.	18	8	—
91	M.M.	20	15	+
	M.M.	20	39	+
92	S.T.	29	4	—
93	F.I.	22	15	+
94	N.O.	21	11	+
95	H.R	21	15	+
96	S.E.	28	14	+
97	K.A.	5	24	+
	K.A.	5	63	+
98	E.R.	15	21	+
99	T.C.	23	12	—
100	M.G.	15	13	—
101	G.W.	12	14	+
102	I.I.	6.5	17	+
	I.I.	6.5	84	+
103	W.E.	9	4	+
104	I.I.	6.5	20	+
105	G.P.	4	15	+
106	L.S.	9	13	+
107	C.A.*	27	12	—
108	W.C.	5	17	+
	W.C.	5	46	+
109	D.W.	2	14	—
110	A.R.	28	19	+
	A.R.	28	102	+
111	F.G.	16	28	+
112	E.H.	18	21	+
113	S.G.	3	16	—
114	G.A.	28	13	—
115	B.M.	5	17	+
116	P.E.	22	18	+
	P.E.	22	48	+
117	L.S.	12	15	+
118	S.F.	5	12	—
119	S.E.	7	12	+
120	S.G.	4	4	—
121	W.A.	12	15	+
	W.A.	12	35	+
122	F.M.	6	13	+
123	F.D.	7	14	+
124	F.S.	7	14	+
	F.S.	7	45	+
125	H.M.	12	19	—
126	S.M.	22	12	—
127	H.O.	11	10	+
128	D.E.	7	15	+

* Designates nephritis.
\+ Designates inhibition of hemolysis.
— Designates hemolysis.

TABLE 3.—*Continued.*

Case	Name of Patient	Age	Day of Sickness When Blood Was Examined	Lymph Gland Extract II
129	M.H.	9	17	+
130	W.M.	6	13	+
131	D.C.	19	11	−
132	F.H.	12	16	+
133	H.R.	6	13	−
134	P.E.	6	15	+
135	F.F.	8	18	+
136	S.M.	4.5	12	+
137	J.E.	4	11	−
138	M.A.	7	24	+
	M.A.	7	69	+
139	M.J.	12	20	+
140	M.A.	10	21	+
141	T.M.	8	11	+
142	McC.J.	27	14	+
143	W.M.	26	15	−
144	L.E.	16	13	+
145	L.J.	11	24	+
146	S.F.	13	21	+
147	T.L.	8	19	−
148	O'B.J.	10	17	+
149	L.J.	22	14	−
150	L.L.	17	24	+
151	C.M.	29	19	+
152	A.R.	4	16	−
153	W.M.	23	27	+
154	C.F.	5	23	+
155	D.G.	17	21	+
156	M.B.	8	18	+
157	W.S.	4	24	−
158	W.F.	14	12	+
159	H.R.	5	14	+
160	M.E.	22	17	−
161	S.L.	12	25	+
	S.L.	12	38	+
162	B.L.	9	21	+
163	M.L.	3	18	−
164	F.G.	13	17	−
165	V.G.F.	17	19	+
	V.G.F.	17	34	+
166	V.G.W.	15	20	+
167	L.F.	11	17	+

* Designates nephritis.
\+ Designates inhibition of hemolysis.
− Designates hemolysis.

In respect to the time of first appearance of the antibodies, it may be said that they do not seem to be present in the blood in demonstrable qúantities before the onset of the second week, the eighth day being the earliest in which positive reaction was obtained. So far as the duration of the action is concerned, the serum was found to be active in the 12th week, and in one case of scarlet nephritis even in the 16th week.

The activity of the extracts gradually diminishes; when in March our work was interrupted, to be begun again in May, the

lymph-gland extract formerly so active had lost its antigenetic properties, and a new extract (II) had to be prepared.

The following conclusions would seem to be suggested by the results obtained:

1. The serum of scarlet-fever patients contains specific antibodies for an unknown virus.

2. This unknown virus seems to be present especially in the cervical lymph glands.

ON THE RELATION OF ILLUMINATING GAS TO PUBLIC HEALTH.*

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INTRODUCTORY.

Sooner or later students of infectious diseases must give closer attention than they have yet found time to give to those environmental conditions which increase or diminish susceptibility to the various infections. When they do this, it will probably be found that obscure poisonings of various kinds play a large part in diminishing vital resistance and in increasing susceptibility. The facts presented in the present paper may be taken as a contribution to this end, for they show how in urban communities one of the commonest of public supplies—the ordinary gas supply—is today a constant menace to the public health, attended as it is not

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only by numerous fatalities but also by many non-fatal poisonings which signify widespread atmospheric impurity in urban dwellings.

The following discussion deals directly and for the most part statistically with *fatalities* from gas poisoning, but the reader must not for a moment suppose that these alone are of importance. Probably even more important are various obscure consequences of the leakage of illuminating gas in private dwellings, in public halls, and in public streets, which are as yet seldom thought of and even more seldom detected.

In 1885 an article bearing the same title as the present paper appeared in the *Annual Report of the State Board of Health, Lunacy, and Charity of Massachusetts.* It was carefully prepared by the able secretary of the Board, the late Dr. Samuel W. Abbott, and the reason for its appearance at that time was the recent perfection of a simple and convenient patented process for the manufacture of a new kind of illuminating gas, then and since known as "water-gas." This was placing upon the market an illuminating gas easier and often somewhat cheaper to make, and ranking higher in candle power, than the ordinary coal-gas derived by the distillation of bituminous coal, but a gas also much more heavily charged with the well-known poisonous substance, carbonic oxide (CO). Promoters of the new process naturally urged its adoption upon the old and established gas companies, which in some cases began to make use of it, especially for supplementary supplies, but in other cases, particularly if they were already prosperous, refused to have anything to do with it. Advantage was also taken of the water-gas process to form new and competing companies, charters being sought on the promise of lower prices and higher candle power for gas. Attempts were likewise made to buy out at low prices long-established and prosperous companies occupying inviting territory, by threats of invasion and competition through gas of lower price and higher candle power.

In Massachusetts, a comparatively thickly settled and therefore attractive territory for the manufacture and sale of gas, there were in the early 80's many and prosperous gas companies, and these for the most part, under the leadership of the largest—the Boston Gas Company—refused to adopt the new process or to be frightened

by threats of competition into selling out at low prices. Furthermore, when the water-gas interests undertook to obtain charters in Massachusetts for new and competing companies they encountered a formidable obstacle in a statute (enacted in 1880) forbidding the distribution and sale of illuminating gas containing 10 per cent or more of CO. This law it was therefore necessary to have annulled before the new process could be introduced into Massachusetts.

A battle royal for the repeal of the law now began between the older coal-gas companies on the one side, who did not care to pay for and use the new process, or did not desire to sell out to the new companies, or did not want competition, and those newer companies which for one reason or another wished to enter Massachusetts territory and sell and distribute water-gas containing more than 10 and often as much as 30 per cent of carbonic oxide. Popular attention was drawn by the old gas companies to the sanitary aspects of the question, and the battle before long raged fiercely around the question of the public health. Meantime the State Board of Health, Lunacy, and Charity referred the mooted question of the relative poisonous properties of the two gases, coal-gas and water-gas, for investigation to two professors of the Massachusetts Institute of Technology—one, the eminent sanitary chemist, the late William Ripley Nichols, and the other, a physiologist, one of the present authors (W. T. S.). These investigators soon after made a report, based upon extensive experiments upon animals, showing that, as might have been expected, much greater danger exists in water-gas than in the ordinary coal-gas (*Report of State Board of Health, Lunacy, and Charity for 1885*, p. 275). In the same report Dr. S. W. Abbott, then secretary of the Board and an excellent statistician, published the paper already referred to above in which he showed that for the preceding 20 years there had been but four deaths from gas poisoning in Massachusetts and predicted many more if the 10 per cent limit should be abandoned.

Victory in the legislature rested for a time with the older companies. But in 1888 the Gas Commissioners, who had been created in 1885, were empowered to license certain companies to make and sell water-gas for illuminating purposes, and in 1890 the 10 per cent statute was repealed, because meantime the opposition of the older

companies was for one reason or another relaxed, while the State Board of Health (as it was now and had since 1886 been called) made no effective objection. Commercial conditions had changed, and many of the coal-gas companies now wanted the privilege of making water-gas if it suited their convenience. Moreover, water-gas was being widely used in other states without public protest, and when the commissioners recommended the change it was speedily made by the legislature—with what obviously disastrous consequences to the public health we shall see in the present paper. Of the unobserved and perhaps imperceptible consequences such as diminution of vital resistance and increased susceptibility to disease, either constitutional or infectious, we have, and in the nature of the case can have, no exact knowledge. There is reason to believe, however, that here also the consequences, if less disastrous, have been no less real.

MORTALITY FROM ILLUMINATING GAS POISONING IN MASSACHUSETTS: MORE THAN 1,200 DEATHS IN THE LAST 20 YEARS.

It was predicted by the investigators employed by the State Board in 1884 and reaffirmed by Dr. Abbott in 1885, that if water-gas should replace coal-gas in Massachusetts the consequences to the public health would probably be dangerous if not disastrous. Other experts of equal or greater eminence took precisely the opposite ground and even ridiculed the possibility of any such consequences. Among these were Professor C. F. Chandler, of Columbia University, and the distinguished English chemist, Dr. E. Frankland, who stated in a letter read during these hearings: "I have no hesitation in saying that it (water-gas) may be used with safety both in public buildings and private houses. I should be delighted to substitute this pure and powerful illuminating agent for the gas with which my house in London is at present supplied, although it is used in all the bedrooms."

More than a quarter of a century has since gone by and there are now available for study the results of a considerable, though by no means total, replacement of coal-gas by water-gas during a period of about 20 years (1890–1909). The authors of the present paper have accordingly undertaken a careful inquiry to see how

far the predictions referred to have come true. The problem is of course complicated by the fact that in spite of the legislative permission to manufacture and distribute water-gas, this has by no means wholly displaced coal-gas. The results at hand are therefore not such as we might have obtained if the replacement had been complete. Since 1890 some companies have made only water-gas; others, only coal-gas; many have made a mixture of the two, and some have made both intermittently. Still, the broad fact remains that an increasingly larger amount of the poisonous gas, CO, has been distributed since 1890 than before that date and not only absolutely but relatively to the population. The matter is further complicated by the use of illuminating gas for suicide, a subject which requires, and in the present paper will receive, special consideration and discussion.

One good result of the long and active agitation in Massachusetts was that a State Gas Commission had been provided for in 1885. Another was that in 1888 the Gas Commissioners were required to investigate, keep a record of, and report all deaths (or injuries to health) from gas poisoning within the state. From 1888 onward, therefore, we have for Massachusetts a fairly complete report of fatalities and other consequences of gas poisoning, and one probably far better than is possessed by any other state. It is perhaps the only record of this kind existing anywhere. Fortunately we have also, for the same period, the returns of the Medical Examiners concerning deaths from illuminating gas—a body of experts whose opinions possess expert value.

We have taken for study the *fatalities*, only, from gas poisoning. Of the numerous injuries which do not result fatally we have, and can have, no complete record. Some are reported by the Gas Commissioners, but many are never reported at all and many less striking and seemingly transient effects, such as headache or malaise, are neither heard nor even thought of as due to gas poisoning.

As stated above, we have obtained the records of death from gas poisoning in Massachusetts from two sources, namely, the reports of the Gas Commissioners, and the returns of the Medical Examiners. The former are published in the Commissioners'

Annual Reports, the latter in the *State Registration Reports*. The Gas Commissioners' records we owe to a provision of law already referred to, permitting the use of water-gas, but at the same time requiring all gas companies to return to the Commissioners a written report of any death or injury due to gas distributed by the company in question. On the whole this arrangement has worked out fairly well, although in the early years the Commissioners complained with reason that some deaths were not reported. The Commissioners' records begin with the year 1886 and it is interesting to note that poisoning by illuminating gas did not earn itself a separate place in the classification of deaths reported by the Medical Examiners until that same year. The figures of the latter are also available, therefore, since 1886. For data previous to 1886 we have only Dr. Abbott's valuable paper of 1885.

The table now given (Table A) shows side by side the figures derived from the two independent sources mentioned. These data are for the *calendar* year indicated, and include only deaths from poisoning by illuminating coal-gas and water-gas. Deaths from oil gas or acetylene gas and deaths caused by gas explosions, or from burning by gas, have been excluded.

TABLE A.

DEATHS FROM ILLUMINATING GAS POISONING IN MASSACHUSETTS. (1886–1909.)

Year Ending Dec. 31	Medical Examiners' Returns	Gas Commissioners' Returns
1886	5	0
1887	6	0
1888	6	0
1889	5	2
1890	12	10
1891	16	14
1892	28	18
1893	27	25
1894	43	33
1895	31	26
1896	52	51
1897	63	58
1898	78	77
1899	65	65
1900	45	46
1901	37	37
1902	63	62
1903	71	72
1904	61	59
1905	77	72
1906	68	71
1907	147	145
1908	123	115
1909	102	99
Totals	1,231	1,157

The table shows substantial agreement in the two sets of data, especially in the later years. The Medical Examiners' figures are probably the more accurate.

Some of the salient features of this table are the sharp rise beginning in 1890 from a very low previous level and continuing

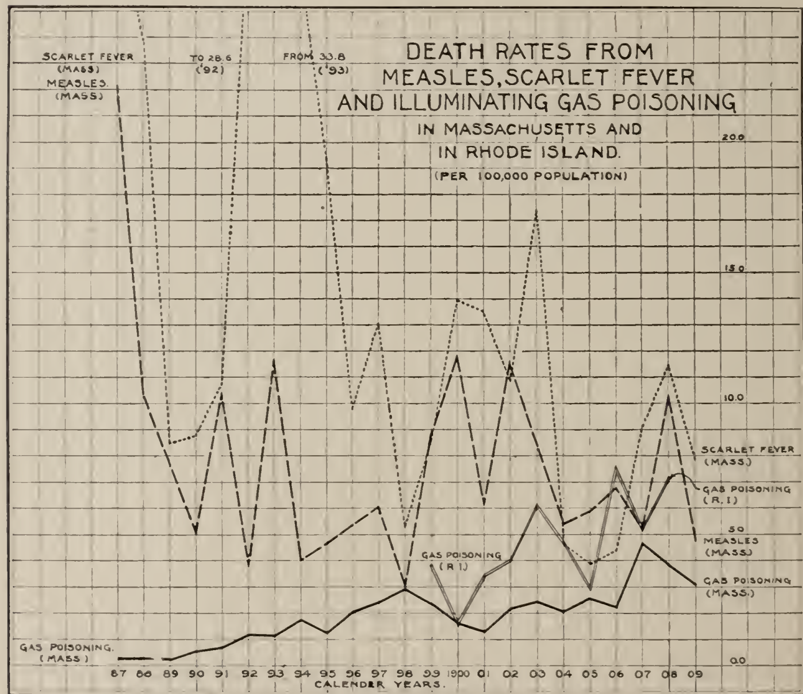

CHART 1.

to a high maximum in 1898, with a fall to a lower level in 1901, followed by a rebound which in 1907 reached the highest point in the entire table. From five or six deaths each year before 1890, the number rose to 147 deaths from poisoning by illuminating gas in 1907. Previous to 1885, according to Dr. Abbott, there had been only four deaths during 20 years.

The same fluctuations which appear in this table may be seen in more graphic form, but as death *rates*, in the lowest curve—the heavy black line marked "Gas Poisoning, Mass."—on Chart 1.

Variations corresponding more closely to those in Table A may

also be seen upon Chart 2 in the heavy black line marked "Deaths by Gas Poisoning." It should be noted, however, that the correspondence of these data is not exact in all cases, since the figures in the table above and the curve on Chart 1 represent *calendar* years and rates, while the heavy black line on Chart 2 represents actual deaths and years ending *June 30*. The highest point of the line on Chart 2 thus falls in 1908 and not as on Table A and Chart 1, in 1907.

SOME DEATH RATES FROM SCARLET FEVER, FROM MEASLES, AND FROM ILLUMINATING GAS IN MASSACHUSETTS AND IN RHODE ISLAND.

We can best realize the growing importance of illuminating gas as a cause of death by comparing its mortality rate for a period of years with the death rates from such familiar diseases as scarlet fever and measles in States having trustworthy vital statistics. For this purpose we have computed the following statistics for two such States; namely, Massachusetts and Rhode Island:

TABLE 1.

DEATH RATES FROM SCARLET FEVER, FROM MEASLES, AND FROM ILLUMINATING GAS IN MASSACHUSETTS AND IN RHODE ISLAND.

(Per 100,000.)

Calendar Years	Scarlet Fever (Mass.)	Measles (Mass.)	Illuminating Gas (Mass.)	Illuminating Gas (R.I.)
1887	28.80	22.08	0.29	
1888	23.78	10.33	0.29	
1889	8.49	7.85	0.23	
1890	8.76	5.09	0.54	
1891	10.74	10.31	0.70	
1892	28.56	3.76	1.19	
1893	33.80	11.52	1.13	
1894	26.51	4.00	1.76	
1895	19.31	4.63	1.24	
1896	9.73	5.35	2.03	
1897	13.05	6.03	2.40	
1898	5.25	2.05	2.91	
1899	8.56	8.78	2.37	
1900	13.94	11.77	1.60	1.63
1901	13.52	6.03	1.30	3.42
1902	10.84	11.53	2.18	4.01
1903	17.42	8.44	2.43	6.10
1904	4.65	5.39	2.06	4.69
1905	3.90	5.89	2.56	1.92
1906	4.39	6.76	2.21	7.50
1907	9.04	5.18	4.67	5.15
1908	11.46	10.27	3.82	7.15
1909	7.86	4.77	3.10	

Table 1 shows strikingly how important poisoning by illuminating gas has recently become as a cause of death in Massachusetts and in Rhode Island; and the same facts are displayed graphically by the diagrams on Chart 1 (p. 386). The death rate from the two contagious diseases (scarlet fever and measles) has evidently greatly

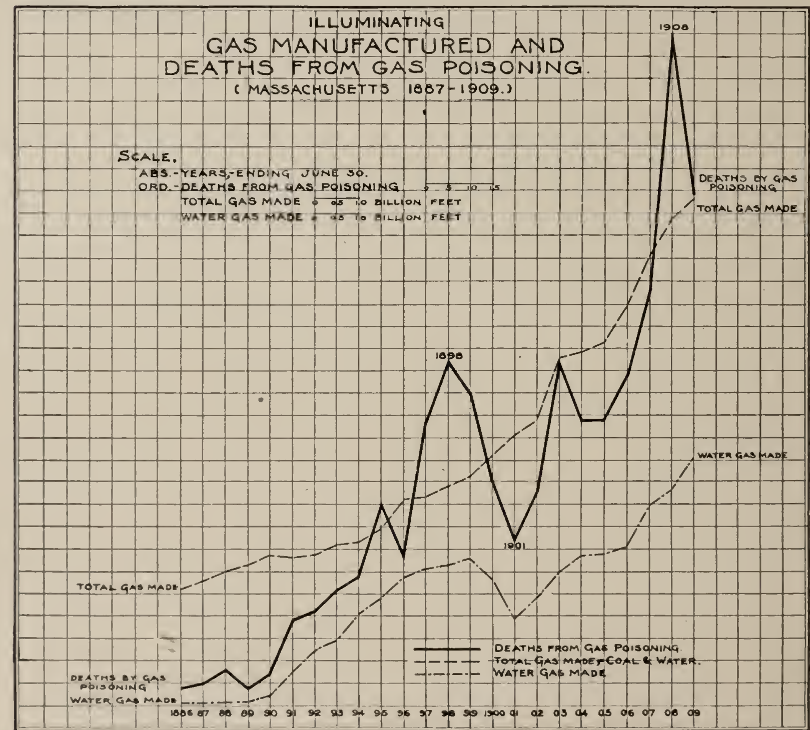

CHART 2.

declined, while that from illuminating gas poisoning has greatly increased, so much so that the latter bids fair soon to exceed the former.

The death rate from poisoning by illuminating gas was higher in Massachusetts in 1907 than from scarlet fever in 1905 and 1906, while in Rhode Island the death rate from illuminating gas from 1903 to 1907 was at times higher than that in Massachusetts from either scarlet fever or measles. Poisoning by illuminating gas has

evidently become in Massachusetts and in Rhode Island a cause of death nearly as effective as are scarlet fever or measles. It has of late years claimed as many victims as has typhoid fever in some American and many German cities.

AMOUNTS AND KINDS OF ILLUMINATING GAS MANUFACTURED IN MASSACHUSETTS (1886–1909).

The following table (Table 2), the main features of which appear also on Chart 2, p. 388, shows the amounts of total illuminating gas (coal-gas and water-gas) and of water-gas, manufactured in each year in Massachusetts. The data are derived from the *Annual Reports* of the Gas Commissioners.

TABLE 2.

AMOUNTS OF ILLUMINATING GAS MADE AND OF WATER-GAS, AND DEATHS FROM ILLUMINATING GAS IN MASSACHUSETTS.

(1886–1909.)

Years Ending June 30	Total Coal- and Water-Gas Made (Million Cu. Ft.)	Water-Gas Made (Million Cu. Ft.)	Deaths from Illuminating Gas
1886	2,625	12	...
1887	2,765	28	5
1888	3,010	47	8
1889	3,156	78	4
1890	3,346	212	7
1891	3,300	777	19
1892	3,370	1,231	21
1893	3,594	1,467	26
1894	3,671	2,022	29
1895	3,955	2,413	45
1896	4,639	2,876	33
1897	4,731	3,090	63
1898	4,901	3,167	77
1899	5,120	3,265	70
1900	5,608	2,881	50
1901	6,059	1,961	37
1902	6,372	2,400	48
1903	7,776	2,989	78
1904	7,882	3,335	64
1905	8,126	3,373	64
1906	8,902	3,536	74
1907	9,998	4,471	92
1908	10,902	4,862	148
1909	11,360	5,518	114

The same facts are depicted graphically upon Chart 2, which deserves and will repay careful study. The apparent discrepancy between these data of deaths and those given on other tables is due to the fact that the "years" end here on June 30, and not as in the other cases on December 31.

A COMPARISON OF MORTALITY FROM ILLUMINATING GAS IN MASSACHUSETTS WITH AMOUNTS AND KINDS OF GAS MANUFACTURED.

From Table 2 and Chart 2 it appears that the total quantity of illuminating gas made in Massachusetts has, on the whole, increased rather steadily, year by year, since 1886. Once only has there been a slight decrease (in 1891) and at times (as in 1896, 1903, 1906, 1907, and 1908) the increase has been very rapid. The curve on Chart 2 shows also on the whole a much more rapid annual increase of output in the later than in the earlier years.

The total quantity of water-gas made shows likewise, on the whole, a great increase since its distribution for illuminating purposes became legally possible in 1890. But the water-gas curve, though approximately parallel to the total gas curve for the years since 1901, was not so before that time. On the contrary, from 1890 to 1896 it was rising much more rapidly; from 1899 it was nearly parallel; and from 1899 to 1901 it declined sharply, whereas the total gas production increased more rapidly than before.

The third line on Chart 2, the heavy black line, shows the deaths, year by year, from illuminating gas in Massachusetts, and, like the other two lines, it shows on the whole a great increase since 1890. It is, however, much less regular in form, and the increase which it shows is much greater than that shown by the other two lines. To the line of total gas production it shows only the most general relation of rapid increase, and that only with numerous and striking exceptions of departure, as in 1896, 1899, 1900, 1901, 1904, 1905, and 1909. If the number of deaths had merely increased *pari passu* with the total amount of gas manufactured, we must have supposed that the poisonous quality of the gas had remained constant and the habits of the consumers unchanged. But this is clearly not the case. The deaths increased very much more rapidly from 1890 to 1898 and from 1901 to 1908 than did the total amount of gas made, while from 1898 to 1901 and from 1903 to 1906 deaths actually decreased while total gas production increased. We are therefore driven to seek some other explanation for the great increase of deaths from illuminating gas than the mere expansion of the industry and the increasing use of gas.

For an explanation we need not look far. If, instead of com-

paring the death curve with the curve of total illuminating gas, we compare it with that of water-gas made, we find a remarkable, though not a perfect, general correspondence. Except in 1896, 1899, 1904, and 1909, this general correspondence is close and striking, both curves rising and falling together, though often at different rates. The general increase in deaths, barring the exceptional years noted, may therefore be readily explained by the general increase in the amount of water-gas made.

From 1898 to 1908 the amount of total gas made had doubled, while the fatalities had not quite done likewise. But while the quantity of total gas made increased about fivefold from 1886 to 1908, the fatalities increased nearly thirty fold. At the same time we find the variations in the amount of water-gas manufactured coinciding much more nearly with the fluctuations in the number of deaths. The remarkable increase of such deaths in 1891 corresponds with the first appearance of any large amount of water-gas. And when the deaths reached a maximum in 1897–99 water-gas had reached a percentage proportion of the total output which it has never equaled either before or since.

In 1900 the New England Gas and Coke Company installed a large coal-gas plant in Everett, and the effect of the introduction of their product into the illuminating gas of the Metropolitan District was to produce an actual decrease for three or four years in the total amount of water-gas manufactured in the state. It is noteworthy and significant that this decrease corresponds closely with the low phase of 1901 in the curve of deaths by gas poisoning. But, as indicated by the diagram, the natural growth of the gas industry soon called for more gas. The check to the production of water-gas in 1901 was only temporary and the increased output since 1901 has been attended by a corresponding increase in deaths from gas poisoning.

In consideration of all these facts we are warranted in concluding that the amount of water-gas produced stands in some close relation to the number of deaths by illuminating gas. This conclusion is justified and confirmed by a comparison of the percentage which water-gas formed of the total gas manufactured, with the deaths per billion feet of total gas produced. If the water-gas is really to

blame, the larger the percentage of water-gas the more dangerous should be each unit of the resultant product. On the other hand, by dividing the deaths from gas poisoning by the total amount of gas made, we should obtain a measure of the poisonous effect of a unit of the total gas. In other words, if the theory that water-gas has been the primary cause of the deaths by gas poisoning is true, we should expect to find some general agreement between the percentage of water-gas to total gas made, and deaths by gas poisoning for each unit, such as a billion feet, of total gas made. That such agreement actually exists appears from Table 3 and its corresponding chart (Chart 3).

TABLE 3.

PERCENTAGE WHICH WATER-GAS MADE WAS OF TOTAL ILLUMINATING GAS MADE, AND DEATHS PER BILLION CUBIC FEET OF TOTAL ILLUMINATING GAS MADE (MASSACHUSETTS, 1887–1909).

Year Ending June 30	Percentage of Water-Gas Made to Total Gas Made	Deaths per Billion Cubic Feet of Total Gas Made
1887	1.01	1.09
1888	1.56	1.17
1889	2.43	0.64
1890	6.34	2.10
1891	22.20	5.76
1892	36.60	6.24
1893	40.80	7.24
1894	55.00	7.90
1895	61.00	11.39
1896	62.00	7.12
1897	65.40	13.31
1898	64.60	15.70
1899	63.80	13.67
1900	51.40	8.92
1901	32.30	6.11
1902	37.70	7.54
1903	38.40	9.78
1904	42.30	8.12
1905	41.30	7.88
1906	39.70	8.31
1907	44.70	9.20
1908	44.50	13.57
1909	48.60	10.05

Table 3, and especially Chart 3, shows a remarkable concordance between the percentage of water-gas manufactured year by year and the corresponding death rate (ratio) from illuminating gas per billion feet of total gas made. In spite of some differences (as, for example, 1896, 1904, 1906, 1908, 1909), it is difficult to avoid the conclusion that the water-gas curve and the death curve stand in the relation of cause and effect.

The agreement between the variations in the percentage of water-gas made and the number of deaths year by year is obviously not absolute, but when we reflect upon the actual conditions under which water-gas is made and distributed, we may well be surprised that the agreement is as close as it is. For illuminating gas is sold in Massachusetts by many companies and under a great

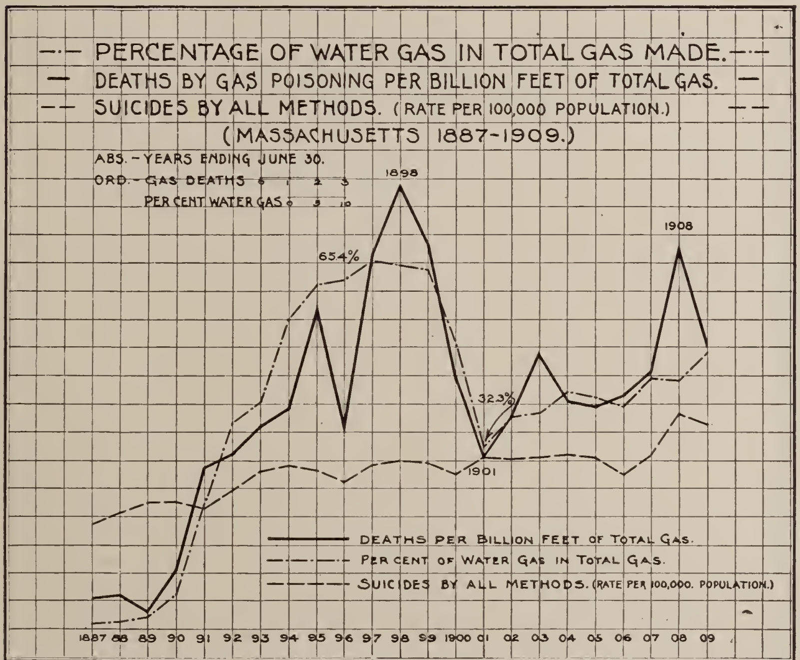

Chart 3.

variety of conditions. Some companies distribute only coal-gas and some only water-gas, but most distribute a mixture of the two. And this mixture may vary widely from time to time in the percentage of the two gases. Again, there is, as we shall learn beyond, a marked seasonal variation in the deaths from illuminating gas, and there is good reason to believe that the mildness or severity of Massachusetts winters may cause annual as well as seasonal variations in the mortality from gas poisoning. These various factors naturally forbid any absolute correspondence between the amount

of water-gas made and the deaths from gas poisoning. When we consider this great variety of circumstances, the wonder is, not that the two curves occasionally differ, but that they run so nearly parallel.

THE USE OF ILLUMINATING GAS IN MASSACHUSETTS FOR PURPOSES OF SUICIDE.

Since 1890 illuminating gas has been gradually discovered by the public to be a convenient and effective means of suicide. Whereas before that time it was very difficult to commit suicide

TABLE B.

DEATHS FROM ILLUMINATING GAS POISONING (MASSACHUSETTS, 1886–1909).
(Medical Examiners' Returns.)

Years Ending June 30	Accidental Deaths	Suicidal Deaths	Total Deaths
1886*	1	1	2
1887	4	1	5
1888	7	1	8
1889	2	2	4
1890	5	2	7
1891	18	1	19
1892	9	12	21
1893	7	9	26
1894	14	15	29
1895	28	17	45
1896	20	13	33
1897	47	16	63
1898	48	29	77
1899	35	35	70
1900	35	15	50
1901	11	26	37
1902	9	39	48
1903	47	30	77
1904	29	35	64
1905	41	23	64
1906	35	39	74
1907	41	51	92
1908	55	93	148
1909	43	71	114
1909†	23	31	54
Totals	624	607	1,231

* First six months. † Second six months.

by the use of illuminating gas, and probably very few would-be suicides resorted to its use, it has come of late years to be one of the easiest and surest agents of self-destruction. The reports of the Medical Examiners contain ample evidence of this fact.

Table B, prepared by us from the returns of the Medical

Examiners, shows not only the use, but the increasing use, of illuminating gas for purposes of suicide. At the same time this table is a sufficient answer to those who have the assurance to proclaim that, excepting as it is used by suicides, water-gas is no more dangerous to life than is coal-gas.

Of the 1,231 deaths by gas poisoning reported by the Medical Examiners in the years 1886–1909, 607, or 49.4 per cent, were

TABLE 4.

Death Rates from Poisoning by Illuminating Gas and from Accidental and from Suicidal Poisoning by Illuminating Gas, (Massachusetts, 1887–1909).
(Medical Examiners' Returns.)

Year Ending June 30	Gas Deaths (Totals)	Gas Accidents	Gas Suicides
1887	0.251	0.19	0.5
1888	0.381	0.33	0.05
1889	0.180	0.09	0.09
1890	0.31	0.22	0.09
1891	0.831	0.79	0.04
1892	0.690	0.33	0.51
1893	1.034	0.71	0.38
1894	1.184	0.57	0.61
1895	1.804	1.12	0.68
1896	1.129	0.78	0.51
1897	2.40	1.79	1.61
1898	2.87	1.79	1.08
1899	2.55	1.28	1.27
1900	1.78	1.25	0.54
1901	1.30	0.39	0.91
1902	1.66	0.31	1.35
1903	2.63	1.61	1.03
1904	2.16	0.98	1.18
1905	2.13	1.36	0.77
1906	2.41	1.14	1.27
1907	2.92	1.30	1.62
1908	4.60	1.71	2.89
1909	3.46	1.31	2.16

reported by them to be suicides and the remainder accidental deaths. Table B gives these data in detail for the separate years.

We have also computed, for the same period, the death *rates* from illuminating gas and those by illuminating gas from accidental sources and from suicidal sources, as reported by the Medical Examiners (Table 4 and Chart 4). It is hardly necessary to repeat that these, while obviously open to the objection that they represent merely the opinion of the Medical Examiners, are the best data we have and are probably on the whole not far wrong.

It may, of course, be urged that it is often difficult even for expert medical examiners to discover whether or not a particular

death was suicidal or accidental. But even if this be granted and if some deaths reported as accidents were really suicides, the reverse may likewise be true, and there is no good reason to doubt that in a large percentage of cases the Medical Examiners' returns are correct. If any reasonable doubt could exist as to the fact that many accidental deaths do occur from poisoning by illuminating

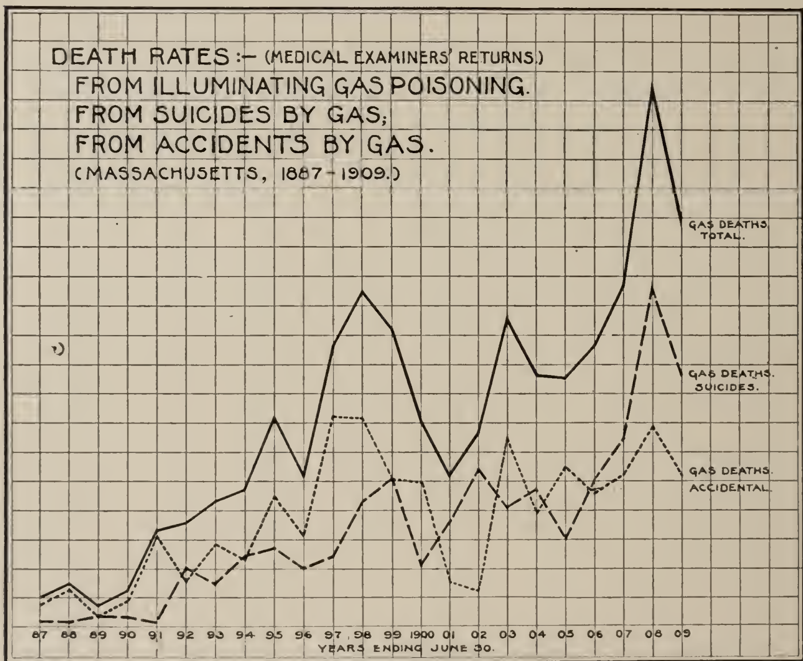

CHART 4.

gas it would be dissipated by an examination of the data shown on the following table (Table 5) and its corresponding chart (Chart 5).

This table and its corresponding plate shows how sudden was the increase in 1891 of deaths from illuminating gas, an increase much more reasonably explained by increase in accidents than by increase in suicidal use of the new and as yet generally unknown poison, especially when we observe that this increase was accompanied by a decrease in the whole number of suicides for the year. Again, in 1895, with no increase in the whole number of suicides, there was a very large increase in the number of deaths from poison-

ing by illuminating gas; in 1904, while the whole number of suicides was increasing, deaths from illuminating gas decreased; while in 1906 the reverse was the case. Undoubtedly, there is on the whole

TABLE 5.

Deaths from Suicide by All Methods, Deaths from Illuminating Gas, and Population (Massachusetts, 1887–1909).

(Medical Examiners' Returns.)

Year Ending June 30	Suicides by All Methods	Deaths from Illuminating Gas	Population of the State
1887	152	5	
1888	175	8	
1889	196	4	
1890	202	7	2,238,943
1891	194	19	
1892	231	21	
1893	270	26	
1894	284	29	
1895	282	45	2,500,183
1896	269	33	
1897	304	63	
1898	321	77	
1899	323	70	
1900	312	50	2,805,343
1901	347	37	
1902	350	48	
1903	358	77	
1904	369	64	
1905	366	64	3,003,680
1906	338	74	
1907	390	92	
1908	494	148	
1909	476	114	

a striking correspondence in the forms of the two curves, such as ought to exist when we remember that (as shown in Table B) about one-half of all the deaths in the lower line are an important factor in the upper.

A STUDY OF THE SEASONAL DISTRIBUTION OF DEATHS FROM ILLUMINATING GAS IN MASSACHUSETTS.

We had not been studying the general subject of illuminating gas poisoning very long before it became plain that such poisoning bears a close relation to the seasons. And this relation proved to be almost precisely what might have been anticipated. Deaths from illuminating gas are comparatively few in summer and comparatively many in winter, as is shown by the first column in the following table, and by the heavy black line on the corresponding chart

(Chart 6) based upon it. The reason is, of course, because in summer, with open windows, short nights, and outdoor life, people in Massachusetts are much less exposed to gas poisoning than in winter, when they are housed most of the time, often in apartments

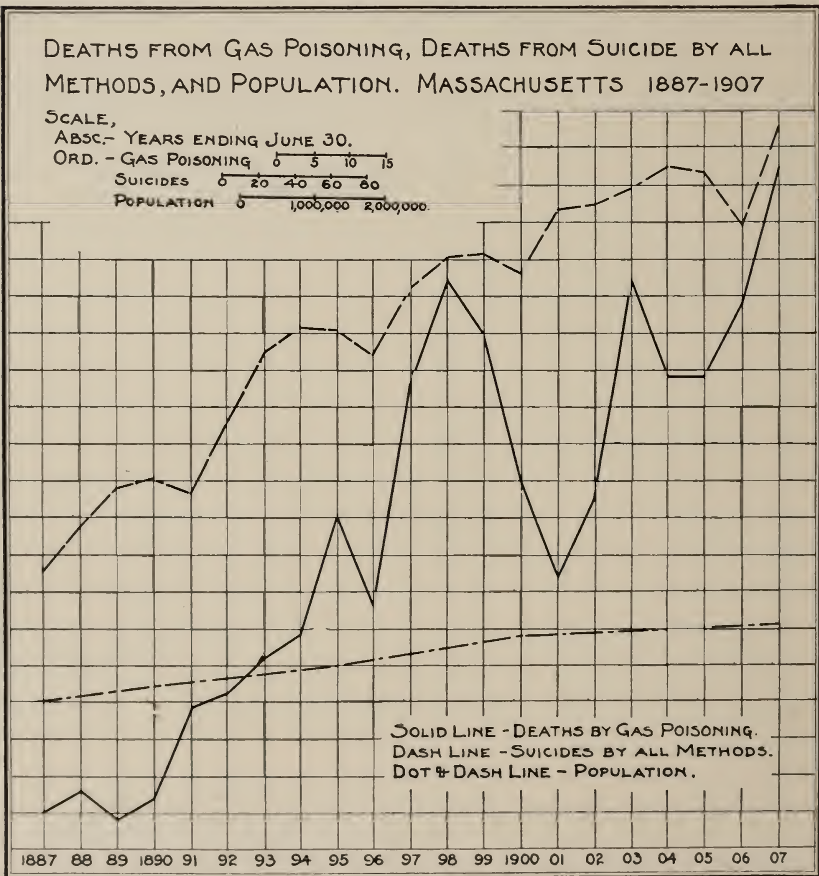

Chart 5.

piped for gas and having little or no ventilation. Similar considerations probably make gas poisoning also largely a matter of latitude—northern cities suffering more from gas poisoning than southern cities—and likewise produce annual variations according as the winters are mild or severe.

This table and the corresponding chart (Chart 6) disclose many interesting and important details. December stands out as the month of most deaths, and December is one of the months of shortest days and longest nights as well as of lowest average temperature. It is therefore one of the times of greatest use of gas, of most indoor life, and of least ventilation by open doors and

TABLE 6.

Seasonal Distribution of Deaths from Illuminating Gas, of Deaths by Accident from Illuminating Gas, of Deaths by Suicide from Illuminating Gas, and of Suicides by All Methods (Massachusetts, 1886–1909).

(Medical Examiners' Returns.)

Month	Total Deaths from Illuminating Gas	Accidental Deaths from Illuminating Gas	Suicidal Deaths from Illuminating Gas	Suicides by All Methods
January	106	73	33	568
February	97	49	48	462
March	106	59	47	597
April	116	51	65	749
May	90	36	54	686
June	67	30	37	647
July	57	20	37	634
August	64	23	41	603
September	92	34	58	611
October	144	77	67	655
November	143	80	63	594
December	149	92	57	541

windows. It is not surprising that these conditions are correlated with the highest mortality from poisoning by illuminating gas. What is remarkable is that the deaths from this source were almost equally numerous in October and November, although the days are then longer, the average temperature considerably higher, and the possibility of comfortable sleeping with more open windows is much greater; while January, which in length of day and temperature closely resembles December, shows fewer deaths than does December from illuminating gas.

July, as might be expected, shows the smallest number of deaths (57), June (67) and August (64) more, and each about the same number, while September yields an increase of more than 40 per cent over June and over August. These facts are not surprising when we reflect upon the cooler and longer nights of June and August over those of July, and the much cooler nights—often with frosts—of September over those of June and of August. But beginning with October there is no great difference in the deaths

by months until we come to January, when in spite of very cold weather and very short days we find a marked decrease of deaths from poisoning by illuminating gas, the deaths for January, February, March, April, and May differing astonishingly little.

We are aided in explaining these various figures by the fact that April and October are the months of most numerous suicides, not

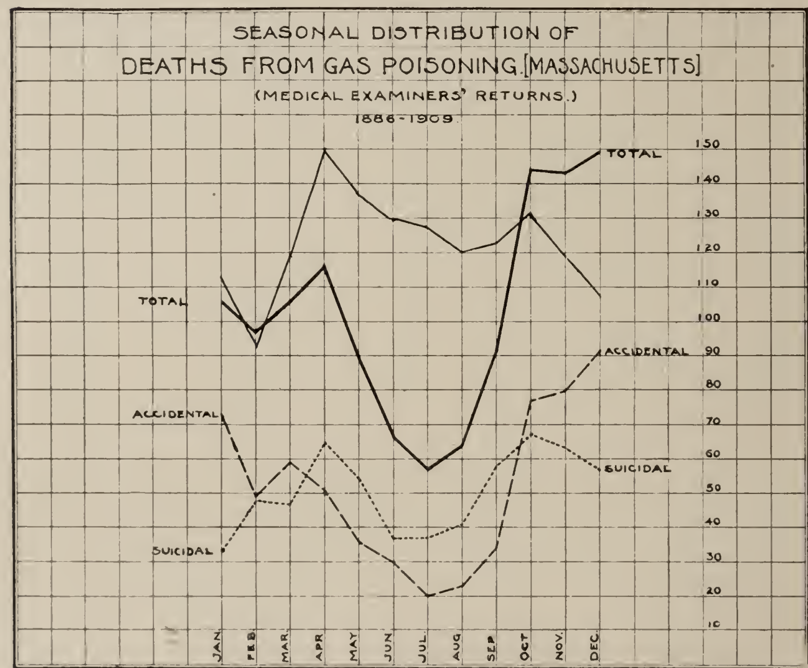

CHART 6.

only by all methods (as shown by the last column in Table 6 and by the corresponding, thin, solid line, without legend, on Chart 6), but also by illuminating gas; so that the curve of total deaths by illuminating gas is necessarily quite different from what it would be if it represented only those deaths due to accident, and if accidents were solely due to considerations correlated with the movement of the seasons—such as temperature and length of days—and effective ventilation. We find here, readily enough, a satisfactory explanation of the large number of total deaths in October (144) and November

(143) as compared with December (149) when the deaths from suicide (both by all methods and by gas) were passing during these months from a maximum to a much lower level. The fact appears to be that while the accidental gas deaths were increasing during this quarter (as appears in Table 6 and Chart 6 and as would be required by theory) the suicidal gas deaths were declining almost *pari passu;* so that a remarkably even and high total of deaths from illuminating gas was maintained during this last quarter of the year.

In the next quarter (January–March) the gas suicides were considerably fewer, as were also the deaths from accidental gas poisoning, and very likely the same explanation holds good of both these causes of death; namely, that conditions had now become comparatively endurable, those that were absolutely intolerable having already destroyed their victims or having been changed for the better. The high number of total deaths in April appears to be chiefly due to the excess of suicides in general characteristic of that month, as does also the lower but still large number in May, when, as required by theory, the seasonal conditions do not greatly favor accidental gas poisonings and when, in fact—as shown by our figures—such poisonings are comparatively few.

It is also interesting to note, in passing, that upon Table 6, and excepting in the first quarter, a close correspondence is shown between the frequency of suicides by all methods and those by illuminating gas.

THE RELATION OF CERTAIN COMBUSTION PRODUCTS OF ILLUMINATING GAS TO THE PUBLIC HEALTH.

The principal combustion products of illuminating gas are carbonic acid (CO_2), water (H_2O), light, and heat. Small, but not insignificant amounts of ammonia, sulfurous acid, soot, and other substances are also produced.

Carbonic acid, an inevitable product of all complete combustion of carbon compounds, while not a desirable addition to the atmosphere of a dwelling, a store, or a workshop, is probably, unless present in very large quantities, of but little consequence from the standpoint of health or comfort.

Water vapor, which is also copiously and inevitably produced in the ordinary combustion of illuminating gas, is probably of more importance than is carbonic acid to health and comfort. Evidence of its abundant presence may often be seen in the water upon the windows of shops, of stores, or of living rooms, upon which it condenses freely and runs down, sometimes almost in streams. The high humidity to which this testifies is often prejudicial to the comfort, and probably also to the health or working capacity, of the inmates.

The light produced by illuminating gas varies widely in amount and composition. The old-fashioned coal-gas gave an agreeable and apparently powerful yellowish light, and when those who were accustomed to it began to be served with water-gas, many found the latter bluish and less efficiently luminous. Much here depends, no doubt, upon the special form of burner, or "tip" employed, but after all is said and done there are many—of whom the senior author is one—who, having lived with both kinds of gas (and with many kinds of mixtures of the two) would gladly go back to the old-fashioned coal-gas at double the present cost of gas, not only because of its greater safety, but also because of its greater and more agreeable illuminating effects.

As to the question of heat produced by combustion—a question of great economic importance for those using gas as a source of power, or for cooking or heating purposes, and of much hygienic significance for persons occupying rooms lighted by gas, it should be said that water-gas is not greatly superior to coal-gas, while, since much heat is produced by the combustion of either gas, their hygienic effects in this particular are probably not very different. Electric lighting (although open to other objections) is vastly preferable to gas lighting on the score of heat production and that of objectionable chemical products.

Among the less abundant products of the combustion of illuminating gas is sulfurous acid. Most coals used in the manufacture of gas—and hence known as "gas coals"—contain a small percentage of sulfur, some of which appears in illuminating gas as hydrogen sulfide and some as bisulfide of carbon, or other sulfur compounds. These, when burned, form sulfurous acid, an irritating and poison-

ous gas. Most of the sulfur compounds in illuminating gas are, however, removed by processes of purification during manufacture, but owing to the difficulty of complete removal, 20 grains of sulfur in every hundred cubic feet have generally been allowed by law to remain in the gas distributed to the public. The 20-grain limit has prevailed in Great Britain for about half a century, and was apparently copied into American statutes when legal regulation of the quality of illuminating gas was first undertaken—in Massachusetts, for example, in 1861. And until quite recently no objection to this legal limit has been raised by the gas manufacturers or by anyone else. A few years ago, however, the London gas companies sought to have this sulfur regulation removed, claiming that because the best gas coals are now scarce, it is much more difficult than formerly to procure coals low in sulfur, so that processes for the removal of sulfur have become more costly and really burdensome to the industry of gas manufacture. And after protracted hearings with the taking of much testimony the sulfur restrictions were, in fact, removed in England. A little later gas manufacturers in the United States and in Canada came forward with a similar demand, and in Massachusetts the legal limit for sulfur has now been raised from 20 grains to 30 grains per 100 cubic feet of gas. A complete discussion of the whole subject of the relation of the combustion products of sulfur compounds in illuminating gas to the public health would require a monograph. Suffice it to say that the testimony taken by the British Commission having the matter in charge, and by the Gas Commissioners of Massachusetts (not to mention other authorities) was voluminous, instructive, and important, and deserving of careful attention. It is the opinion of the authors of the present paper that the British authorities were not sufficiently considerate of the public health aspects of the subject when they allowed all restrictions upon the sulfur content of illuminating gas to be removed, and that the Massachusetts Gas and Electric Light Commissioners acted more wisely when they declined to follow the British example and merely relaxed somewhat the severity of the legal requirements regarding sulfur.

Illuminating gas is required by law in Massachusetts (and in many other places) to be free from ammonia as well as from

sulfuretted hydrogen, but this is more because of injury to fixtures than because of danger to health.

THE PREVENTION OF POISONING BY ILLUMINATING GAS.

The question naturally arises, What can be done for the protection of the public health against poisoning by illuminating gas, which as a cause of sickness and death now almost equals in Massachusetts and Rhode Island some of the dreaded infectious and contagious diseases? We must admit at the outset that about one-half of the recorded deaths from this source are voluntary or suicidal, but while recognizing this fact we have no right to dismiss it as irrelevant to the present discussion. The State seeks, as far as possible, to prevent suicide, by laws, for example, regulating the sale of other poisons and of firearms, and may well regard with concern the general distribution to the public of a dangerous gas readily available for self-destruction.

Even more serious are the public consequences of the widespread distribution to sick and well for industrial and domestic purposes of a dangerous and highly poisonous substance, insidious in its mode of operation, quickly harmful in its effects, and delivered under such pressure that leaks are frequent. Those who have read and reflected upon the facts given in the preceding sections will hardly need to be told how prejudicial to the public health even small leaks of illuminating gas must be, especially if long continued, while the leakage or escape of larger amounts is well known to be often fatal to those exposed. The simplest and most natural remedy for these evils would be, of course, to return to the former practice of making and distributing only coal-gas instead of water-gas or a mixture of the two. But here, as so often in public health problems, a balance must be struck between industrial advantages accruing to the public in less cost, and some saving of life and health. If the industrial, economic, or efficiency gain is very great, it may justify some increase of danger to life and health. But if it is not very great, then life and health have the prior claim. In the present case it is not claimed that water-gas in Massachusetts or Rhode Island is, as a rule, much if any cheaper to manufacture than is coal-gas, but that it is very convenient to produce because

more quickly made when needed. The claims of the advocates of water-gas in 1884 that this gas would furnish 24 candles of light against the 16 candles of the old-fashioned coal-gas do not appear to have been substantiated, since most of the gas now distributed in Massachusetts equals—according to the State Inspector—only about 18 candles. The price of gas to the consumer has, however, fallen greatly since 1884, and this decrease in cost, as far as it is due to the use of water-gas, must be balanced against the damage done to the public health by the loss of more than 1,200 lives and an unknown amount of less obvious injury to life and health.

Undoubtedly a mixture of coal-gas and water-gas, such as is often distributed today, is less dangerous than is water-gas alone, but this appears to be merely because the dangerous constituent, carbonic oxide, so abundant in water-gas, is diluted by the process of mixture; and up to a certain point, the greater the dilution, the less the danger. We know from experience that when carbonic oxide forms only about 6 per cent of illuminating gas very little danger exists. We also know from experience that 20–30 per cent of carbonic oxide means danger. But whether 10 per cent or perhaps 12 per cent might be allowed without much danger, we do not yet know. It should, however, be possible to determine by experiment the minimum amount safely allowable.

Meantime, in view of the appalling loss of more than 1,200 lives which has occurred in Massachusetts since the 10 per cent restriction was removed, it seems not unfair or unreasonable to demand a return to the 10 per cent limit until such time as evidence shall be forthcoming that a higher percentage will properly safeguard the public health.

APPENDIX.

A DIGEST OF LEGISLATION IN MASSACHUSETTS BEARING UPON THE RELATION OF ILLUMINATING GAS TO PUBLIC HEALTH.

Franz Schneider, Jr.

Acts of 1861, chap. 168. *An Act for the Inspection of Gas Meters, the Protection of Gas Consumers, and the Protection and Regulation of Gas Light Companies.*

Section 1. Appointment of inspector. His duties. Sec. 2. His tenure of office; compensation; oath of office; bonds; etc. Sec. 3. List of gas companies. Inspector's salary, how paid. Delinquent companies, proceedings against. Sec. 4.

Appointment of deputies. Qualifications, disabilities, fees, etc. SEC. 5. Standard of measure. SEC. 6. Sealing of meters. SEC. 7. Apparatus. SEC. 8. Test gas-holders. Test meters. Examination, proving and stamping. Record of meters. SEC. 9. Expense of testing meters. SEC. 10. Standard of gas. Twelve candle-power minimum. "Whenever requested by the Mayor and Aldermen of any city or the Selectmen of any town, the inspector shall report to them whether the gas supplied is of the legal standard, and also whether it is sufficiently well purified from sulphuretted hydrogen, ammonia and carbonic acid." SEC. 11. Right of companies to enter premises, etc. SEC. 12. Right to stop gas, etc. SEC. 13. Penalty for injury, trespass, etc. SEC. 14. Fraudulent use of gas. SEC. 15. "This act shall apply to all companies which manufacture gas for sale, and shall take effect July first, eighteen hundred and sixty-one. . . . . Approved, April 10, 1861."

It was under this act that Hon. John A. Andrew, the famous "War" Governor of Massachusetts appointed as the first State Inspector of Gas Meters and Gas, the late Professor William Barton Rogers, founder and first president of the Massachusetts Institute of Technology. No further legislation of a sanitary sort appears to have been enacted until 1880.

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Acts of 1880, chap. 230. *An Act in Addition to "An Act for the Inspection of Gas Meters, the Protection of Gas Consumers, and the Protection and Regulation of Gas Light Companies.*

SECTION 1. Assistant inspector of meters to be appointed by the Governor, etc. SEC. 2. Inspector and assistant to be paid traveling expenses. Sec. 3. Meters to be sealed. Penalty. SEC. 4. Company to provide a suitable room containing a disc photometer. SEC. 5. Inspection of Gas. (See Pub. Stat. 61 [14].) Read "provided" for "but" in sentence 1; "whenever" for "when" in sentence 4. Add to sentence 4: "but no fine for any impurities found before the first day of September, eighteen hundred and eighty shall be imposed"; read "whenever" for "when" in sentence 5. SEC. 6. Amendment of Act of 1861, chap. 168 (7). SEC. 7. Repeal of Act of 1861, chap. 168 (6). . . . . Approved April 22, 1880.

This act of 1880, chap. 230, was introduced in the House on April 1, 1880, for the Committee on Manufactures and passed to second reading; third reading following on April 6; to be engrossed, April 11. It was introduced into the Senate and passed to second reading, April 13. Third reading was refused April 14; reconsideration was requested April 15; passed to third reading April 19; enacted April 22, 1880. These facts are interesting as showing that there was no serious opposition of any kind to the passage of the bill. A search of the files of the *Boston Transcript* for that period fails also to reveal any special interest in the subject and yet it was Section 5 of this Act of 1880 which, as we shall now see, prevented for the next ten years the legal manufacture and distribution of water gas for illuminating purposes.

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Pub. Stat. Mass., Enacted November 19, 1881, to take effect February 1, 1882, Chap. 61. *Of the Inspection of Gas and Gas-Meters.*

SECTION 1. Appointment of inspector and assistant inspector and their terms of office. 1861, 168; 1880, 230. SEC. 2. Salaries, etc. SEC. 3. Bonds. SEC. 4. Inspector and assistant not to be interested in manufacture of gas, etc. SEC. 5.

General duties of inspector and assistant. SEC. 6. Appointment of deputy inspectors. Fees. SEC. 7. Salaries and expenses of inspector and his assistant to be paid into the State Treasury by gas companies. 1861, 168 (3); 1878, 223. SEC. 8. Standard measure for gas. SEC. 9. Apparatus and chemicals provided. SEC. 10. Gas-light companies and vendors of meters to provide test gas-holders and gas-meters, etc. SEC. 11. Meters not to be used unless stamped. SEC. 12. Testing of meters in use. SEC. 13. Gas-light companies to furnish room with photometer. SEC. 14. "The gas of every company supplying more than fifty consumers shall be inspected at least twice a year, and one additional inspection shall be made for every four million cubic feet of gas supplied by each company; but the gas of no company shall be inspected oftener than once a week. All such inspections shall be made by the inspector or his assistant and one-fourth at least of all such inspections shall be made by the inspector. The gas shall be tested for illuminating power by means of a disc photometer, and, during such test, shall be burned from the burner best adapted to it, which is at the same time adapted to domestic use, and at as near the rate of five feet per hour as is practicable. When the gas of any company is found on three consecutive inspections to give less light than fifteen standard English candles, or to contain more than twenty grains of sulphur or ten grains of ammonia per hundred cubic feet of gas, or more than 10 per cent of carbonic oxide, or any sulphuretted hydrogen, a fine of one hundred dollars shall be paid by such company to the city or town supplied by it. When during the test the consumption of gas varies from five feet per hour, or the candle from one hundred and twenty grains per hour, a proportional correction shall be made for the candle power." 1880, 230 (5). SEC. 15. Right of entrance. SEC. 16. Right to stop gas. SEC. 17. Penalty for injury to meter, etc. SEC. 18. For unlawfully using gas. SEC. 19. Companies to which chapter applies.

Under SEC. 14 above, the eight words "or more than 10 per cent of carbonic oxide" entirely prevented the legal distribution of water gas for illuminating purposes in Massachusetts. It was not until 1890 that this restriction was removed.

* * * * * * * * * * * * * * *

Acts of 1885, 240 (1). *Gas allowed for heating, cooking, chemical and mechanical purposes. Exemption granted from Pub. Stat.*, chap. 61, secs. 13 and 14.

Be it enacted . . . . as follows:

SECTION 1. The provisions of SECS. 11, 52 and 75 of chap. 106 of the Pub. Stat. are hereby extended so as to authorize the establishment and operation of corporations for the purpose of making, selling and distributing gas for heating, cooking, chemical and mechanical purposes. Said corporations shall have all the powers and privileges and be subject to all the duties, restrictions and liabilities set forth in all general laws which now or hereafter may be in force relating to gaslight companies; provided, however, that secs. 13 and 14 of chap. 61 of the Public Statutes shall not apply to gas made and used exclusively for heating, cooking, chemical and mechanical purposes. SEC. 2. Such gas shall not be used for domestic purposes unless connected with a chimney or flue having direct connection with the open air; provided, however, that nothing in this section shall be construed to apply to illuminating gas as defined by the provisions of sec. 14, chap. 61 of the Pub. Stat. Any violation of this section shall be punished by a fine not exceeding twenty dollars for each and every offense. SEC. 3 This act shall take effect upon its passage. . . . . Approved May 15, 1885.

Acts of 1885, chap. 31. *An Act to Establish a Board of Gas Commissioners.*

SECTION 1. Board of Gas Commissioners to be appointed by Governor. Also clerk. SEC. 2. Term of office (3 yrs.). Vacancies. SEC. 3. Commissioners forbidden to be interested in sale of gas. SEC. 4. Salaries. SEC. 5. Incidental expenses. SEC. 6. Annual expenses to be assessed from gas companies. SEC. 7. Gas companies to make annual sworn returns. SEC. 8. Board to have supervision of corporations making and selling gas, etc. SEC. 9. Investigation of complaints, as to quality of gas. SEC. 10. Second gas company not to dig up streets without permission. SEC. 11. Purity of gas. May investigate and recommend regulations to the legislature. SEC. 12. Attorney-general must be notified if company neglects to comply with orders. SEC. 13. Provisions may be enforced in equity. SEC. 14. Annual report to legislature. SEC. 15. Gas inspectors to render assistance, etc. SEC. 16. Gas companies may appeal to Board in case of grievance. SEC. 18. Approved June 11, 1885.

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Acts of 1886, chap. 250.

Amends Pub. Stat., chap. 61, sec. 14, to read six million cubic feet instead of four million cubic feet.

* * * * * * * * * * * * * * * *

Acts of 1888, chap. 350. *An Act in Addition to "An Act to Establish a Board of Gas Commissioners."*

SECTION 1. Fixing price. SEC. 2. All companies and individuals engaged in the business of manufacturing and selling gas and electricity for light or fuel shall make a written report within twenty-four hours to the Board of Gas Commissioners of every accident caused by the gas or electricity manufactured or supplied by them, whereby an employer or any other person shall suffer bodily injury or loss of life or be rendered insensible, stating the time, place and circumstances of the accident, and such other facts in relation thereto as the Board may require; and the Board shall present in its annual report an abstract of all such cases. The Board shall personally investigate all cases which it may deem to require investigation. SEC. 3. This act shall take effect on the 30th day of June, 1888. . . . . Approved May 17, 1888.

* * * * * * * * * * * * * * * *

Acts of 1888, chap. 428. *An Act Authorizing the Gas Commissioners to License Certain Gas Companies to Make and Sell Water Gas for Illuminating Purposes.*

SECTION 1. "The Board of Gas Commissioners is hereby authorized to license any company now authorized to make gas for illuminating purposes to make and sell water gas for illuminating purposes containing any percentage of carbonic oxide that said Board may determine: *provided* that such Board shall be of opinion and shall certify in any license granted by them that in their opinion the gas so authorized can be used with safety for such purposes, and after receiving such license said company shall be exempt from any penalty or prohibition provided in sec. 14, of chap. 61 of Pub. Stat. relating to carbonic oxide provided the percentage of carbonic oxide shall not exceed the limit allowed by said Board. SEC. 2. Any company which shall under the provisions of the first section of this act be licensed to make and sell water gas for illuminating purposes containing an excess of 10 per cent of carbonic oxide

shall furnish to every actual consumer a copy of the Gas Commissioners' license which shall contain a statement of the percentage of carbonic oxide such gas contains as near as can be ascertained. And no company so licensed shall charge more for water gas in any locality than is charged in that locality by any company furnishing gas therein when the manufacture and sale of such water gas is so licensed." SEC. 3. This act shall take effect upon its passage. . . . . Approved May 29, 1888.

* * * * * * * * * * * * * * * *

Acts of 1890, chap. 252. *An Act Removing Restrictions from the Manufacture and Sale of Water Gas for Illuminating Purposes.*

SECTION 1. Sec. 14, chap. 61, Pub. Stat. relating to inspection of gas is hereby amended by striking out in the fifteenth line the words "or more than 10 per cent of carbonic oxide," so that the last two clauses of said section as amended shall read as follows: "When the gas of any company is found on three consecutive inspections to give less light than fifteen standard English candles, or to contain more than twenty grains of sulphur or ten grains of ammonia per hundred cubic feet of gas, or any sulphuretted hydrogen, a fine of one hundred dollars shall be paid by such company to the city or town supplied by it. When during the test the consumption of gas varies from five feet per hour, or the candle from one hundred and twenty grains per hour, a proportionate correction shall be made for the candle power. SEC. 2. Chap. 428, Acts of 1888, authorizing Gas Commissioners to license gas companies to make and sell water gas for illuminating purposes is hereby repealed. SEC. 3. This act shall take effect upon its passage. . . . . Approved April 30, 1890.

* * * * * * * * * * * * * * * * *

Acts of 1892, chap. 67. Raises candle power standard required from 15 to 16 standard candles.

Acts of 1902, chap. 228. (1) Transfers powers and duties of inspector of gas meters, etc., to Gas and Electric Light Commissioners.

Acts of 1903, chap. 464. Amends section providing for inspection and testing of illuminating gas. Modifies methods but not substances to be tested for.

THE MANKATO TYPHOID FEVER EPIDEMIC OF 1908.*

H. M. Bracken, F. H. Bass, F. F. Wesbrook, H. A. Whittaker, and H. W. Hill.

(From the Minnesota State Board of Health, St. Paul, Minnesota.)

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GENERAL INTRODUCTION.

H. M. Bracken.

The description of the outbreak of this epidemic is well stated by Dr. A. O. Bjelland, then commissioner of health of Mankato, as follows (*Transactions*, Minn. State Sanitary Conference, 1908):

"In the year 1908, there was an unusually heavy rain-fall in and about Mankato, beginning May 20 and lasting until June 24, causing a tremendous flood. The Minnesota River reached its highest point June 25 and 26, the highest water point at Mankato since 1881. The sewers were unable to take care of the immense amount of water, and hence the cellars along Front and Second Streets were flooded."

About June 26 diarrhea became epidemic throughout the city, 5,000 or 6,000 cases occurring between this date and July 4.

"Dr. H. M. Bracken (secretary and executive officer of the State Board of Health) was advised by telephone of the above conditions, and was asked to aid in making an investigation. The mayor (Dr. J. W. Andrews) was notified on June 27 of the probability that the public water supply was contaminated, and the people were warned through the daily papers to boil the city water to be on the safe side.

"On June 27, an investigation of the Broad Street well was started. On June 28 (Sunday) there was a meeting of Mayor Andrews, Judge Porter, Mr. Koke, Mr. Atchison, Mr. Gary, and Dr. Bjelland, at which matters were discussed, the health

* Received for publication September 11, 1911.

officer advising those present that an epidemic of intestinal trouble was prevailing, and that an epidemic of typhoid fever might follow.

"On June 29, Dr. H. M. Bracken, Dr. H. W. Hill, Mr. Haynes, the city engineer, Mr. Koke, Judge Porter, and myself (Dr. Bjelland) made an investigation of the well-pits and reservoirs."

Dr. Bracken, acting for the State Board of Health, officially announced that the circumstantial evidence found, together with the epidemiological facts connected with the diarrheal trouble, indicated the city water as responsible, and predicted a possible typhoid-fever epidemic, in the event of typhoid bacilli being contained in the contaminating sewage. Samples of water were taken for confirmatory analysis.

On July 1, Professor F. H. Bass, sanitary engineer of the State Board of Health, investigated the wells, and later made more detailed studies, with recommendations for changes and repairs.

"At this time I ordered the Third Street well shut off, the reservoir cleaned, and further, that the reservoir and water mains be flushed until further notice. The only thing that could be done at this time to safeguard the health of the people was to advise everyone to boil the water until further notice. It was evident, that since the infection had been introduced into the water, nothing else could have been done at that time to protect the public."

(Purification by hypochlorite of lime was not then known. Other disinfectants were advocated by various persons, but no one knew how much to use or the attendant dangers and drawbacks.)

"On July 12 the result of the bacteriological examination of the water was given, showing positively that there had been sewage contamination in the water mains, the presence of the colon bacillus being demonstrated.

"Early in July, Mrs. B. developed symptoms indicating the possibility of typhoid fever. A specimen of blood was submitted to the State Board of Health Laboratory for examination, and on July 11 a positive Widal reaction was reported."

These statements of Dr. Bjelland as to the beginning of this epidemic are most interesting. There was some difficulty and delay in persuading some of the officials of Mankato of the actual danger, for they were not satisfied to base action purely on the inspection and the epidemiological facts, delaying until the Laboratory made its analytical report. Another noticeable feature is that typhoid was tentatively predicted.[1]

This epidemic differs from others in that it was anticipated instead

[1] Previous (and subsequent) experience in other water-borne outbreaks of dysentery and diarrhea emphasize the fact that typhoid fever by no means always follows such outbreaks.

of being taken up after the disease had become epidemic, the first case actually being discovered, diagnosed, and reported, and the epidemic followed through from this time to its termination.

The State Board of Health not being equipped properly at the time for a continuous epidemiological study in such an epidemic, Dr. H. W. Hill, assistant director of the laboratories of said board, was put in charge of the epidemiological work, following the custom that had been in vogue in dealing with special epidemics up to that time. Professor Bass, who was then acting as sanitary engineer for the board, took up the work from the engineering point of view. Both of these gentlemen made frequent trips to Mankato for the study of conditions during the epidemic.

An emergency hospital was secured, and the Sisters of the Sorrowful Mother, who maintained the St. Joseph Hospital, furnished the nurses for it.

On July 24, Mr. M. C. Piper, a senior student in the Medical Department of the university, began work as assistant to the health commissioner, and local agent of the State Board of Health, visiting each case reported, collecting the epidemiological data, and making a daily systematic inspection of the typhoid-fever areas. Early in August, Miss Jessie Clark and Miss Grace Robinson were engaged as visiting nurses. It was the duty of these nurses, as soon as the report of a typhoid-fever case came in, to go to the house and instruct the patient and his friends how to avoid infection by contact, to teach the family thoroughly in the use of cleanliness and antisepsis, and by an object-lesson in nursing show those in charge how to care for the patient.

Due credit is to be given the local workers already mentioned. The work of the State Board of Health was largely carried out by Dr. H. W. Hill, representing the epidemiological side, and Professor F. H. Bass, representing the engineering side. Mr. John Wilson was secured at Professor Bass's suggestion to perform the function of city engineer. Following the epidemic Dr. G. A. Alley was sent out as special agent of the State Board of Health to get as complete a history as possible of all typhoid-fever cases and deaths occurring outside of Mankato traceable to this source of infection. His data is included in the report of Dr. Hill.

A thorough publicity campaign, begun early, was a most important feature of the work. The co-operation of the local press, the *Free Press* and the *Review*, in spite of the opposition of some of the business interests, prevented much primary infection, and was a large factor in keeping secondaries within unusually low bounds. Mankato owes much to the public-spirited newspapers.

The reports of the various workers are appended.

May 13, 1911.

THE ENGINEERING DATA.

F. H. Bass.

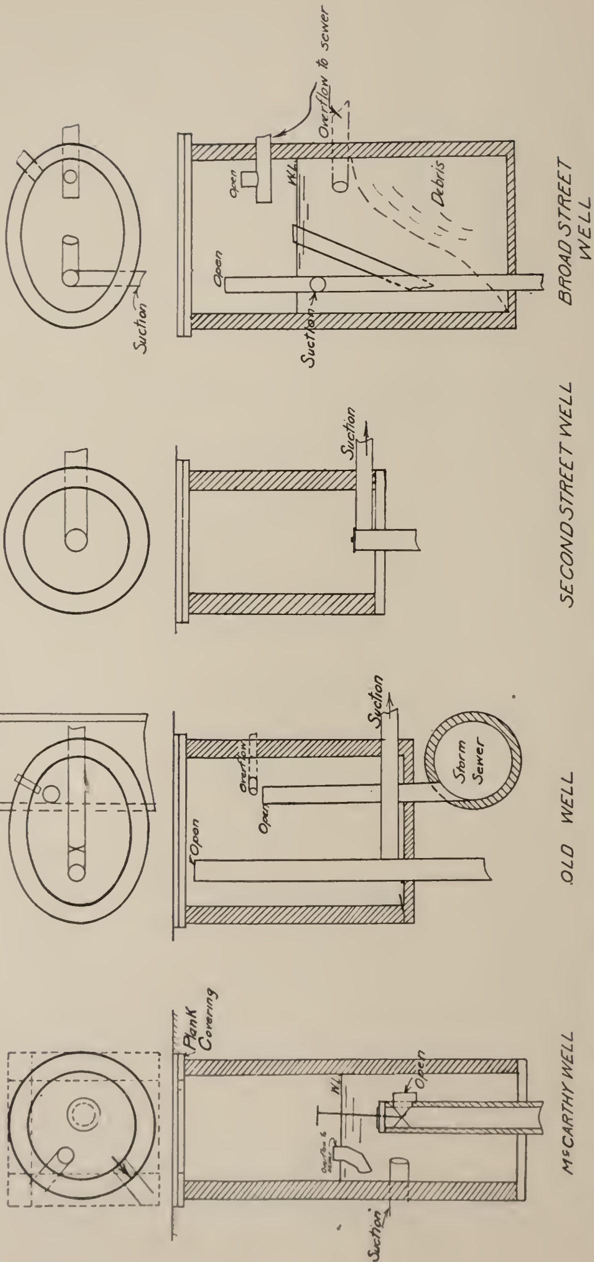
The engineer of the State Board of Health made an examination of the wells and their relation to possible sources of contamination, beginning July 1, 1908.

Heavy rains in the early summer had caused the highest water in the Minnesota River since 1881. The crest of the flood was reached June 25, and on this date there occurred in Mankato an extremely heavy rain, the run-off of which, from the precipitous slope immediately east of the city, flooded several streets to a depth of over one foot for their entire width. On one of these streets, Washington Street, are located all of the wells from which the city supply was drawn. The wells with their various connections are shown in the upper part of Fig. 2, p. 418.

Fig. 1 shows these wells in some detail. The McCarthy well was of recent construction. The "old well" was sunk some 20 years ago, and both the overflow to the sewer and the suction pipe were badly corroded. Holes of 1.5 inches in diameter entirely through the pipe were found in both. I saw one of these plugged with a poorly fitting piece of wood. The sewage from the sewer immediately beneath this well-pit certainly backed up into the pit and as surely was drawn through the holes in the suction pipe to the pumps and distributed.

As the Second Street well was of recent construction and in good condition no contamination from it was possible.

The Broad Street well was found in a condition of extreme neglect. When first found by the writer, this well-pit was filled with water to a point within four feet of the surface of the ground,



(Minnesota State Board of Health Engineering Division: Compiled from drawings furnished by Mr. John Wilson, City Engineer of Mankato, Minn.)

FIG. 1.

and the lower outlet to the sewer was dimly visible through the still turbid water. It was obvious from the débris in the crevices between the brick side walls and plank covering that the water in the pit had been as high as the surface of the ground.

A curiously placed pipe in an inclined position, which appeared to be a branch of the well-casing, occupied the center of the pit. No one in Mankato could tell what this pipe was, although many guesses were hazarded; later a workman stepped upon it and it rolled to one side. This incident is mentioned to bring out the remarkable fact that previous to the epidemic absolutely no one was familiar with the construction of this well. Later, upon the subsidence and clarification of the water in this pit, it was found that the supposed top of the well-casing was in reality but one end of the vertical arm of a T, the other end terminating a foot or more above the bottom of the pit. It is absolutely certain that the sewage backed up into the pit. Near-by manholes show that the sewage backed up to within 18 inches of the surface of the street, or about six feet above the bottom of the pit. Thus sewage was pumped directly into the water mains of the city.

At the outlet of the sewer passing the Broad Street well was a large manhole or pump-pit, divided into two parts by a wall at right angles to the line of the sewer. In the upstream division of this pit was a small centrifugal pump which at the time of high water pumped sewage over the top of the wall. At ordinary stages of water in the river, the sewage flowed directly through a gate in the wall. At the time of this flood, the pump was unable to handle the large quantity of sewage coming to it, hence the backing up into the system of pipes in the city.

The engineer of the pumping station was notified of the appearance of sewage in the engine rooms of a near-by flour mill by an attendant at the latter place. The gate in the dividing wall of the well was opened and the danger was supposed to be over. However, the unusual appearance of the city water supply was seen and the president of the Board of Public Works was notified by the health officer of the city. He refused to stop the pumps, as there was not sufficient water in the reservoir for emergency in case of fire.

When the pumps were not running, it was customary to allow the overflow of the Broad Street well to run through the suction-pipe, which sloped downward toward the pumps, through the pumping station to the river. There is some uncertainty as to the exact time when the gate in this pipe was opened, but during a certain period the sewage was high enough on the lower side of the gate to allow it to flow back into the suction-main in considerable volume. There is evidence to show that this must have occurred, since the sewage backed up to the boiler-room floor, and its route thence was through a manhole into which the above-mentioned suction-pipe discharged.

None of these conditions making possible the contamination of the water supply were known to the City Council, to the Board of Public Works, to the Health Commissioner, or to anyone else.

Mankato citizens prided themselves on their supply of pure water, but their assurance was founded on mere a ssumption, and a terrible calamity resulted.

No one in particular was to blame; the form of government which divided the responsibility and the lack of appreciation of the need of care in obtaining and maintaining proper sanitary conditions was the underlying cause.

Such bad conditions now probably exist in more than one Minnesota city. The Engineering Division of the State Board of Health should be in a position to examine regularly all sources of water supply. Such inspection is at least of as much importance to life and property as the inspection of steam boilers, which is regularly carried on.

RECONSTRUCTION.

A double object was held in view in making repairs, both to make the pollution of the water impossible and to increase the capacity of the supply.

The first step was to rebuild the "old well." This well was of 8-inch bore with a 10-inch casing to the rock at a depth of 130 feet. The well was reamed out to a diameter of 10 inches; an 8-inch casing with a rubber packer was placed inside of the 10-inch casing and a tight joint made with the rock. An old uncompleted

well was also found at the manhole. At the surface it was fitted with a goose neck extending above the highest possible high-water level in the river and then run to the river to provide for any occasional overflow.

The second operation was to determine the exact location and condition of suction-mains. These were found to be overflows or drains from the wells to the pump rather than true suction-mains; the pumps would not operate until there was about three-pounds pressure from the wells, hence the full capacity of the wells could not be realized.

All pipe near the pumping station was found to be of wrought iron, and in bad condition. There were many holes extending through the pipe, and several joints entirely rusted through, and the main from Broad Street to the pumping station had many leaks. This old main was torn up and relaid on a grade of 0.10 per cent upward toward the pumping station. Flanged pipe of 12 inches in diameter from the pumping station to Front Street, and 10 inches in diameter from Front Street to Broad Street was used. The old cast-iron pipe from the pumping station to Front Street was in good condition, but from Front Street to Broad Street there were found 28 breaks, and in two places the pipe had broken off entirely. In one break an attempt had been made to mend the pipe by wrapping canvas around it. A cold chisel could be driven through the pipe at one blow.

The connection to Second Street well was lowered to increase the flow and a water-tight manhole was built.

Broad Street well was reconstructed. The old bore hole could not be exactly located because the well casing had been long since corroded. A length of old smoke stack had been put in around this casing, and evidently packed with flax seed. Also, a length of cast-iron pipe which had evidently been used in an attempt for repairs, was found. After making numerous attempts to locate the well in the rock 90 feet below the surface, a new well was put down. A 20-inch pipe was put down into the rock to a depth of four feet, and a 10-inch pipe was put inside of this. The space between these two pipes during construction was filled with fine sand. Eighty-six cartloads were used in all. The water during the sink-

ing of this well was coming up on the outside and the purpose of putting the sand inside was to allow it to pass down around the lower end of the pipe and be carried up by the water to stop the flow on the outside. This expedient succeeded; after a while the water came up inside the 10-inch pipe, which was then driven to a depth of four feet below the 20-inch pipe. Finally, the space between the two pipes was filled with cement mortar, making a cement pipe which would always be in place after the destruction by corrosion of the iron pipes. A concrete manhole was constructed around this well. The bottom was left open and covered with sand so that if the old well should by any chance break out it would come up through the manhole and make itself evident. The reconstructed wells are shown in Fig. 2.

After this, the suction-main on the McCarthy well was lowered and cast-iron pipe substituted for the wrought-iron pipe.

Changes in the suction-pipes in the neighborhood of the pumping station were made as indicated in Fig. 3.

After this, the cross connections from the main sewer to the drain from the pumping station were cut off.

Lastly, a new sewage pumping station was built and in it were installed an 18-inch centrifugal pump geared to a 65 H.P. motor, capacity 10,000 gallons per minute, and an 8-inch pump geared to 15 H.P. motor, capacity 1,000 gallons per minute. Both of these pumps were designed to operate against a 20-foot head. This new construction is shown in Fig. 4.

All of the reconstruction was under the direct charge of Mr. John Wilson, city engineer of Mankato, a graduate of the Engineering College of the Minnesota State University.

The location of wells, suction-mains, and sewers on Washington Street is shown in Fig. 5.

Fig. 6 is a map prepared to show graphically the extent to which the epidemic spread outside of the city of Mankato.

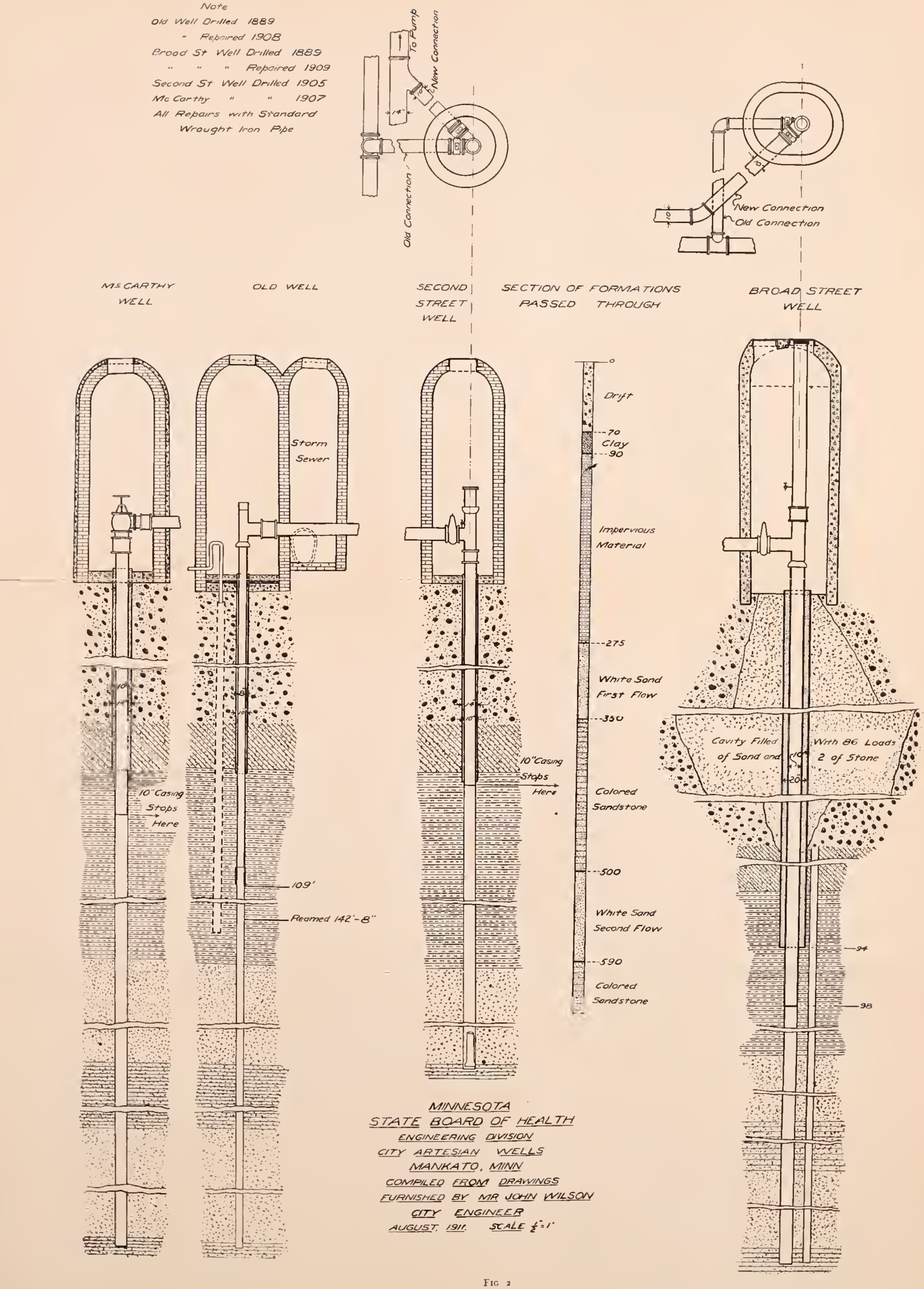
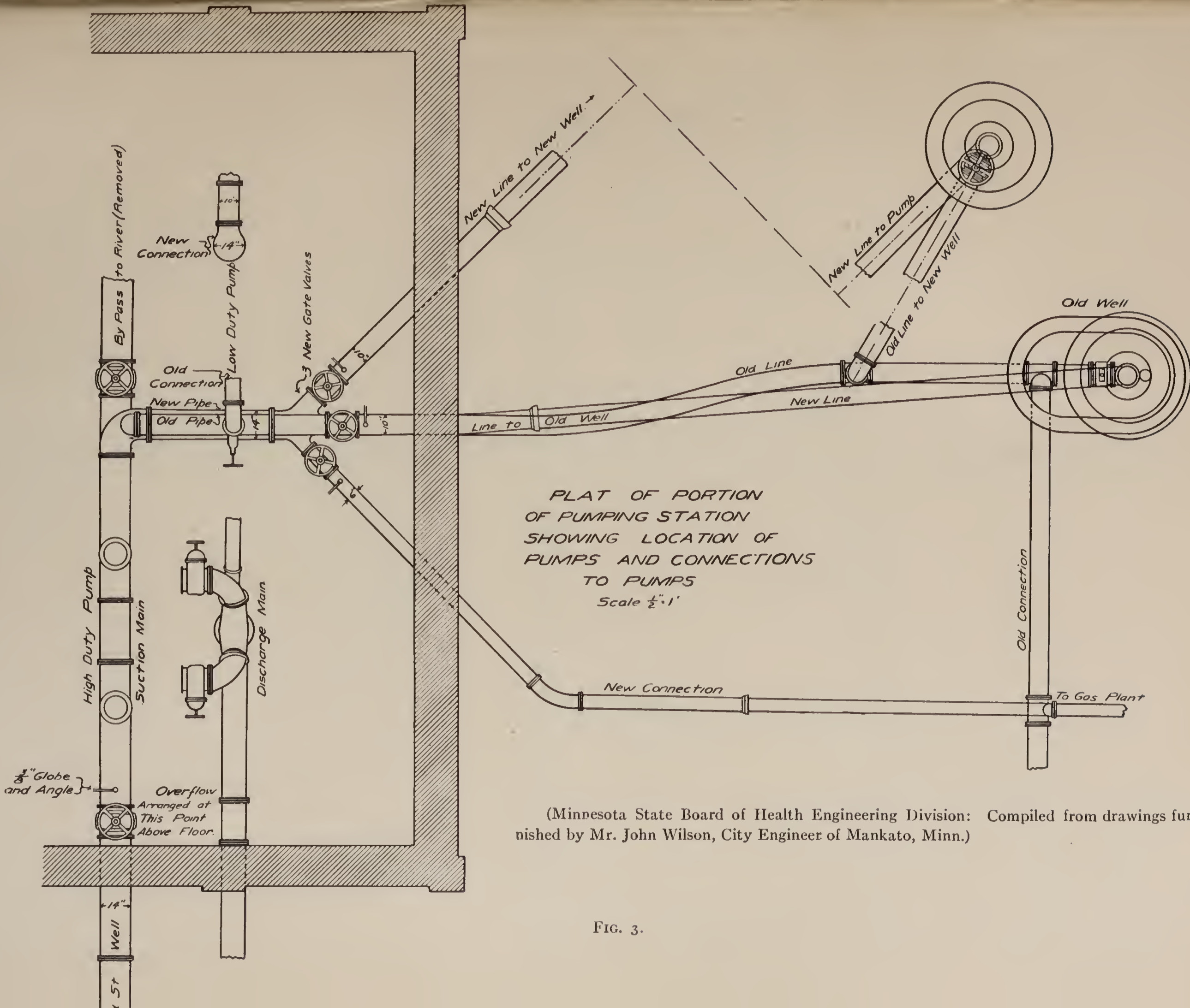
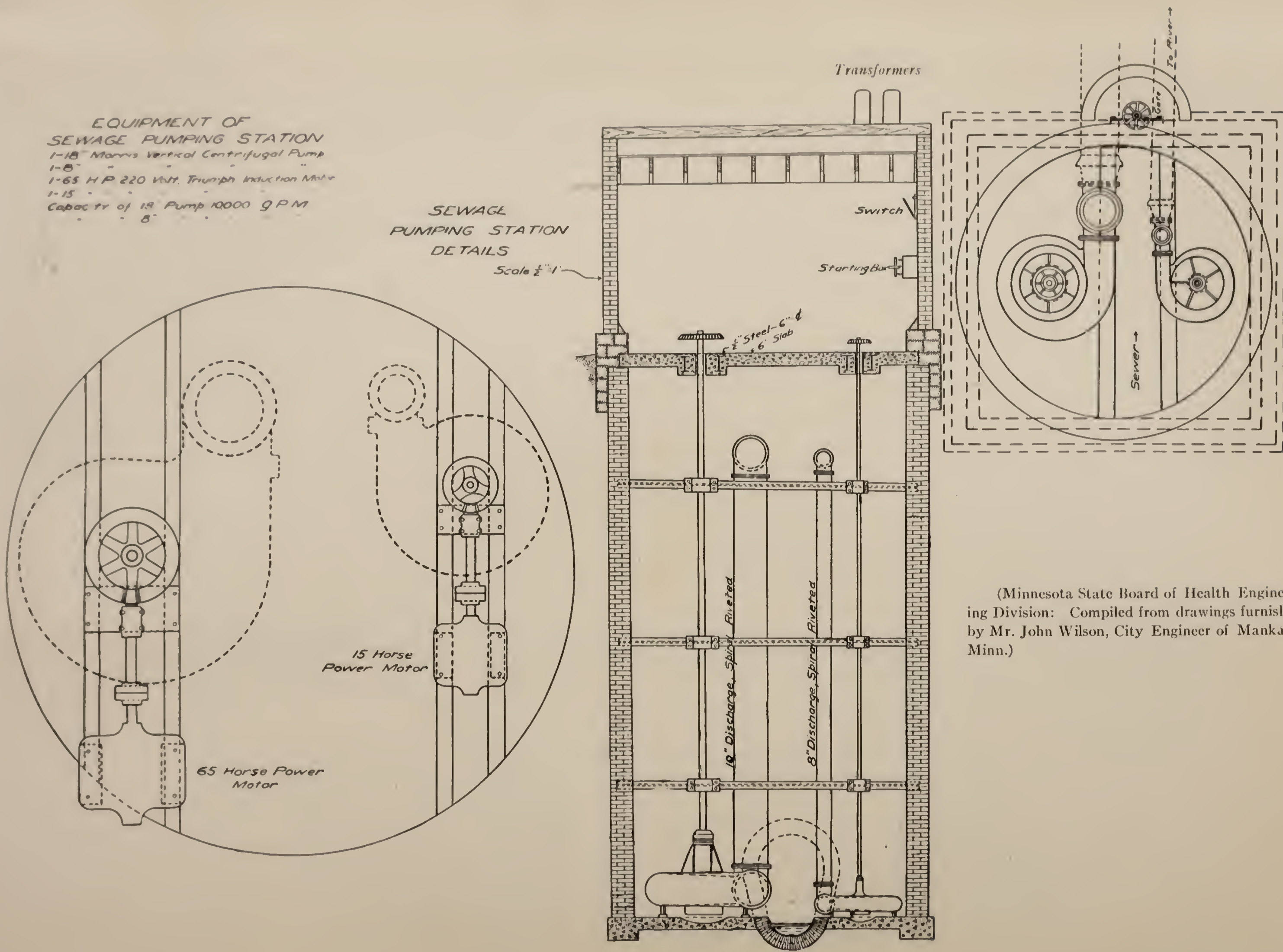

Note
Old Well Drilled 1889
" Repaired 1908
Broad St Well Drilled 1889
" " " Repaired 1909
Second St Well Drilled 1905
McCarthy " " 1907
All Repairs with Standard
Wrought Iron Pipe
To Pump
New Connection
Old Connection
New Connection
Old Connection
McCARTHY WELL
OLD WELL
SECOND STREET WELL
SECTION OF FORMATIONS PASSED THROUGH
BROAD STREET WELL
Storm Sewer
Drift
70
Clay
90
Impervious Material
275
White Sand First Flow
350
Colored Sandstone
500
White Sand Second Flow
590
Colored Sandstone
10" Casing Stops Here
10" Casing Stops Here
109'
Reamed 142'-8"
Cavity Filled of Sand and With 86 Loads 2 of Stone
94
98
MINNESOTA
STATE BOARD OF HEALTH
ENGINEERING DIVISION
CITY ARTESIAN WELLS
MANKATO, MINN
COMPILED FROM DRAWINGS
FURNISHED BY MR JOHN WILSON
CITY ENGINEER
AUGUST. 1911. SCALE ½"=1'

Fig 2



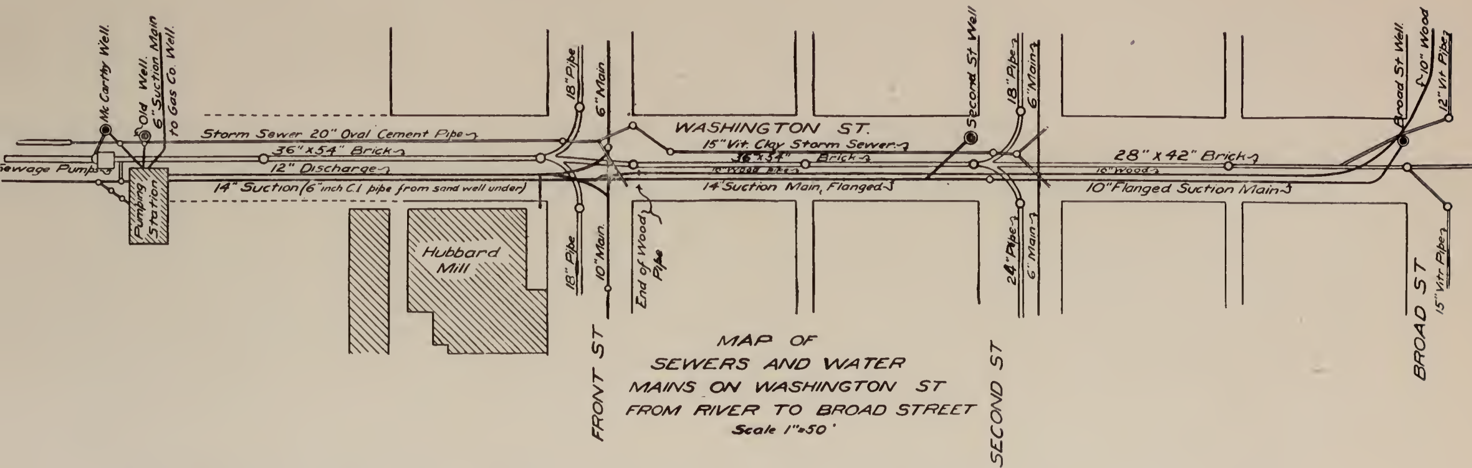
(Minnesota State Board of Health Engineering Division: Compiled from drawings furnished by Mr. John Wilson, City Engineer of Mankato, Minn.)

Fig. 3.



(Minnesota State Board of Health Engineering Division: Compiled from drawings furnished by Mr. John Wilson, City Engineer of Mankato, Minn.)

FIG. 4.



(Minnesota State Board of Health Engineering Division: Compiled from drawing furnished by Mr. John Wilson, City Engineer of Mankato, Minn.)

FIG. 5

THE LABORATORY DATA.

F. F. Wesbrook and H. A. Whittaker.

The epidemiological investigation and the resultant field and administrative work were by far the most important and interesting phases of the Mankato outbreak. During the Mankato work, the time and attention of Dr. H. W. Hill, at that time Assistant Director of the Laboratories, were required for months, and a large share of the laboratory work was thrown upon the remaining members of the laboratory staff.

These points appear to be worthy of mention, since there were many lines of work which very naturally suggested themselves at the time and since, which have had to remain uninvestigated in favor of more pressing problems whose solution might be of immediate practical service. Since then, the epidemiological division of the Minnesota State Board of Health has been established with Dr. H. W. Hill as director. The credit for the epidemiological work done in connection with this epidemic justly belongs to Dr. Hill, and it has seemed only proper, therefore, that the report be presented and discussed in its epidemiological relations by him, particularly as Dr. Hill was responsible for both epidemiological and field laboratory work in Mankato at that time. On this account, and because the Mankato studies were of epidemiological and engineering rather than of laboratory interest, the tabulations of bacteriological, chemical, physical, and other water examinations, Widal examinations, examinations of blood, excreta, etc., will be omitted.

LABORATORY WORKERS.

The selection of field problems and collection of materials during the active work in Mankato were in the hands of Dr. H. W. Hill. The collection and forwarding of samples of water and of much valuable data, including sketches and information in relation to water and sewage conditions, have been most admirably and thoroughly carried out by Mr. John Wilson, after his appointment as city engineer of Mankato. Dr. A. J. Chesley, late chief of the Diagnostic Laboratory of this division and now an epidemiologist to the Minnesota State Board of Health, took charge of the routine bacteriological examinations of water, excreta, blood, etc., in addition to much other important work, while Mr. M. G. Roberts,


MINNESOTA
STATE BOARD OF HEALTH
ENGINEERING DIVISION
MAP OF MINNESOTA SHOWING LOCATION OF NON-RESIDENT TYPHOID CASES CONTRACTED IN MANKATO, 1908
SCALE OF MILES
0 5 10 20 30 40 50
On Farms Near Municipality Indicated
In " "
In Mankato but residents of " "
TOWNER ND
RYDER, ND
COLUMBUS ND
SEATTLE, WASH
WHITE SD
STAPLES
HOLLOWAY
WILLMAR
MINNEAPOLIS
REDWOOD FALLS
FAIRFAX
MORGAN
NEW ULM
SPRINGFIELD
CAMBRIA
JUDSON
LAKE CRYSTAL
SOUTH BEND
RAPIDAN
VERNON CENTER
AMBOY
TRUMAN
NEW PRAGUE
LE SUEUR
OTTOWA
ST PETER
KASOTA
NICOLLET
MANKATO
WATERVILLE
EAGLE LAKE
JANESVILLE
ST CLAIR
OWATONNA
DECORIA
GOOD THUNDER
MAPLETON
NEW RICHLAND
EASTON
WELLS
AUSTIN
JACKSON
BRICELYN
BIGELOW
MANSON IOWA
CECILIA MICH
WINONA

Fig. 6.

chemist, undertook the chemical examinations. Later Mr. H. A. Whittaker has conducted all of the laboratory examinations of water, bacteriological, chemical, physical and biological, and has made a special study with Mr. John Wilson of the sources of pollution of the Mankato water system. He has also collected, collated, and tabulated all of the laboratory data available for Mankato, both routine and special, before, during, and since the outbreak. Acknowledgment should be made to Dr. O. McDaniel, chief of the Pasteur Institute of this division, for oft-repeated and laborious assistance at times when the work was pressing.

WATER INVESTIGATIONS.

In this report reference will be made only to examinations for Mankato and vicinity which bear directly upon the epidemic of 1908. For those who are interested in the details of the bacteriological and chemical findings of the surface waters and information concerning the well-waters, reference should be made to "Water Supply and Irrigation Paper No. 193," issued jointly by the United States Geological Survey and the Minnesota State Board of Health, authors R. B. Dole and F. F. Wesbrook. See also United States Geological Survey "Water Supply Paper No. 256," published by the Survey in co-operation with the Minnesota State Board of Health, authors C. W. Hall, O. E. Meinzer, and M. L. Fuller. See, also, *Bulletin No. 154*, Department of Agriculture, Bureau of Plant Industry, by Karl F. Kellermann and H. A. Whittaker, in co-operation with the Minnesota State Board of Health, which gives certain references to the Mankato epidemic and analyses of water which relate to the cases cited in the bulletin as of Mankato origin.

The analyses of all samples of water collected at Mankato during the period discussed have been prepared in the form of analytical tables, but are omitted for the sake of brevity.

Eighty-six samples of the Mankato public water supply were examined from June 29, 1908, to August 15, 1910,[1] while 26 samples

[1] All examinations were conducted in accordance with the Standard Methods promulgated by the American Public Health Association. The plates and colon sowings were made in the field according to the methods of these laboratories (*Jour. Infect. Dis.*, Supplement 1, 1905, p. 321; *Bull. No. 154*, U. S. Department of Agriculture). Mr. Wilson, who has had a long experience in laboratory water and sewage work and who is thoroughly familiar with the Minnesota methods, prepared the plates, etc., in the field when the laboratory representatives were not present.

of school, private wells, or other supplies were made for various purposes. The four wells comprising the city supply were located within the flooded area, and data collected in the field showed opportunity for the infection of three of them. The four wells were the McCarthy well, the Old well, the Second Street well, and the Broad or Third Street well. (For details of operation, structure, and environment, as also for stratigraphic data, see the Report of the Engineering Division, which precedes this, and also United States Geological Survey "Water Supply Papers Nos. 193 and 256.")

PUBLIC SUPPLY BACTERIOLOGICAL EXAMINATIONS.

Eighty-six examinations were made in which the number of bacteria per c.c. varied between one and 200, except in three samples, where it reached 425, 1,500, and 6,600, respectively.

June 29, 1908, the count ranged from 50 to 200 colonies per c.c., with an average of 130 per c.c., colon bacillus appearing in the Third Street well, and in the system in 1 c.c. amounts and in McCarthy well in 100 c.c. amounts.

July 18, 1908, the count varied between 25 and 35 bacteria per c.c., and colon bacilli were not found present in one c.c., and were doubtful in McCarthy well, but were easily demonstrated in 100 c.c. amounts in the Third Street well and from a tap on the system (mixed wells).

August 22, 1908, the count varied between five and 30 with an average of 17 c.c. Colon bacilli were found present in only one of six samples, and then only in 100 c.c. amounts in a sample from the system, where the count was five bacteria per c.c.

September 25, 1908, the count varied between 16 and 120, averaging 40 per c.c. Colon bacilli appeared in 1 c.c. amounts in only one of the five samples, the count for this sample being 16. It was present in only one other sample in 100 c.c. amounts, the count for the sample being 27, and the high count sample (120) not showing colon bacilli at all.

October 5, 1908, the count ranged from two to 36 bacteria per c.c. with an average of 11 per c.c., colon bacilli not being found present either in one or 100 c.c. amounts in five samples.

October 22, 1908, the count ranged from four to 10 bacteria per c.c., the average being six colonies per c.c. Colon bacilli were found in two of the five samples in 100 c.c. amounts, but were not found in 1 c.c. amounts in any of the samples.

November 9, 1908, the count ranged from one to 425 bacteria per c.c., colon bacilli not being found present in any of the four samples in either 1 or 100 c.c. amounts.

December 14, 1908, the counts varied between one and seven per c.c., the average of the six samples being less than three per c.c., and colon bacilli were not found in any of the samples, even in 100 c.c. amounts.

January 14, 1909, the counts ranged from 5 to 28 per c.c., averaging 17 per c.c., and colon bacilli were reported in only one sample in 100 c.c. amounts.

February 8 and March 17, 1909, somewhat similar results were obtained, colon bacilli being present only in 100 c.c. amounts, but occurring in six of the nine samples, the counts ranging from four to 25 bacteria per c.c., the average being 11 per c.c.

April 7 and May 10, 1909, colon bacilli could not be demonstrated even in 100 c.c. amounts in any of the nine samples, the counts ranging from three to 25 bacteria per c.c., the average being 12 per c.c.

On August 5 and November 2, 1909, 11 samples were examined and colon bacilli reported in 100 c.c. amounts in five samples, the counts of which were 4, 10, 20, 12, and 11 bacteria per c.c., while the six other samples showed no colon bacilli even in 100 c.c. amounts, although the count for one sample was 1,500 per c.c.

May 16, June 13, and August 15, 1910, samples showed no evidence of colon bacilli in wells or system.

The laboratory examinations also revealed the presence of a large number of molds in samples collected from the infected sources and the system. It seems reasonable to take this as additional evidence of infection.

These analytical data corroborate the field inspection and point out infection in three of the wells and in the system.

The laboratory examinations after July 29, 1908, showed a gradual disappearance of the infection, as indicated by the disappearance of colon bacilli in small amounts, a marked lowering in the bacterial counts and the disappearance of the molds above mentioned.

It is true that colon bacilli appeared from time to time in 100 c.c. amounts in samples collected from the system, yet the significance of this is not great when it is remembered that repairs and changes were made in the system from time to time after the original infection. Mr. John Wilson, who later was appointed city engineer, has carefully observed conditions in the water system in Mankato since the epidemic, and has furnished the following explanations relative to the possible sources of colon bacilli present in 100 c.c. amounts in the city water from time to time since the marked infection disappeared.

"In the first place, all suction-mains from the Old well and the McCarthy well were completely removed and replaced the last week in December of 1908, and the first week in January of 1909. This also necessitated a complete change of all piping inside the pumping station.

"Such work might logically introduce colon bacilli, which would appear in small numbers for some time afterward.

"In connection with the appearance of colon bacilli in 100 c.c. amounts in samples taken from the Broad Street well, repairs on this well were completed about July

15, 1909. As will be noticed from the cross-section of the well (in repiping), we failed to locate the old well, or the original hole in the rock. It is reasonable to suppose, however, that we were very close to it, although it would hardly seem possible that the two intersect. At least the results of my experiment would point that way. The cavity around the well was filled with the finest grained sand obtainable and taken from the river bank. No doubt the original hole itself was filled with this material, in which case the water entering the new hole from the side of the original hole would pass through this sand, which, from its source, would be highly impregnated with colon bacilli, of which a number might be carried through the thin wall separating the two holes. The final disappearance of the organisms would seem to indicate that the above might be a correct solution of the problem.

"The appearance of colon bacilli in small numbers in samples taken from the house taps, while samples taken from the sources failed to show it present, was a perplexing problem. It occurred to me that the condition of the reservoir might throw some light on the matter.

"The reservoir is built of stone, about one-half of which is above ground, and banked up with earth on the outside. It is covered with a wooden roof, in the top of which is a latticed ventilator. The doors are also partly composed of screens. Within 50 feet of the reservoir is a small barn, where I found that the owner had allowed manure to accumulate. Our health officer had this manure removed, believing that the wind might possibly carry contamination through the ventilator and screens.

"The hillside below the reservoir has always been in a more or less of a springy condition and I suggested that much of this might have its source from the reservoir. It was explained that this could not be the case, as the red iron stain, characteristic of the city water, was absent. However, upon emptying the reservoir, it was found that there were a large number of minute openings, following the joints in the stone walls, through which the water was seeping back into the reservoir. It would seem that this might be a more potent factor in introducing colon bacilli into the supply than the ventilators, as the water would seep out when the reservoir was full, and be drawn back when the reservoir was low, bringing with it some of the organisms from the surrounding soil.

"It is interesting also to note that our reservoir and surrounding soil acted as a very efficient iron removal plant. The entire interior of the reservoir was plastered and all leaks stopped, during the past summer (1910).

"The two abnormal counts given on our list are hard to explain, and the only possible solution I can suggest is that the taps from which the samples were taken were not sufficiently flushed to yield a fair sample. These taps are controlled by a common ¾-inch brass angle valve. I have, of late, in addition to flaming the tap with the alcohol lamp, opened and closed the valve a number of times in order to be sure that all sediment or foreign material has been removed before taking the sample. The above may not be a very satisfactory explanation, but it is the best I can offer at the present time."

The data collected by Mr. Wilson furnish an explanation of the source of colon bacilli recurring in the wells after the original infection had apparently disappeared, and indicate possible routes

for those colon bacilli which were found spasmodically in the distribution system. The two abnormal counts mentioned are undoubtedly due to some such condition as described, for they are unusual and no other explanation can be suggested. The material collected by Mr. Wilson and the epidemiological data make it appear that this occurrence of colon bacilli in 100 c.c. amounts in the later examinations had little if any sanitary significance.

The epidemiological data go to show that typhoid infection from water supply was a temporary affair. The bacteriological examinations at the time served as a basis for such an epidemiological prognosis, yet the occasional presence of colon bacilli precluded the possibility of giving the water a clean bill of health. Had the efficiency of hypochlorites been recognized then as now, a sterilization of the system would have afforded an opportunity of determining the significance of the infrequent appearance of colon bacilli. From all available data it would appear justifiable to regard the occasional presence of *B. coli* as due to repeated local infection during reconstruction, etc., rather than to its prolonged persistence after the initial gross pollution of the whole system.

The unexcelled opportunities for observation in many directions which are now so plain, would have warranted, among other investigations, a search for *B. typhosus* in the water at the time of gross pollution with colon bacilli, particularly since the total bacterial count was relatively so very low. As already stated, however, the facilities and workers of the laboratory were overtaxed in numerous other directions.

PUBLIC SUPPLY PHYSICAL AND CHEMICAL EXAMINATIONS.

Complete sanitary physical and chemical analyses were made of Mankato public supply between June 29, 1908, and August 15, 1910, on 55 samples. The results have been tabulated by one of us (H. A. W.), but space considerations forbid their introduction here. In order, however, to give some idea of the quality of the water the following statement may be useful. The table below was compiled from 25 analyses of samples of water collected from various points on the distribution system between June 29, 1908,

and August 15, 1910. These samples represent the mixed water from the various wells connected with the public supply.

	Average	Minimum	Maximum
Turbidity	17	2	60
Color	11	tr.	38
Total hardness	310	208	395
Alkalinity	329	312	528
Incrustants	21	0	81
Ammonia (albuminoid)	0.062	0.028	0.146
" (free)	1.216	0.520	2.280
Nitrites	0.003	0	0.020
Nitrates	0.002	0	0.040
Chlorine	7.06	3.00	30.00
Iron	2.38	tr.	9.60

The marked variations noted are largely due to the different amounts of water drawn from the individual sources from time to time and the normal fluctuations in the quality of the underground waters.

PRIVATE SUPPLY BACTERIOLOGICAL EXAMINATIONS.

During the water investigations at Mankato, 26 wells not connected with the city supply were examined. These were private supplies, some of which were contemplated for temporary public use, and industrial, school, and private wells, etc. Of this number, 65.3 per cent showed no laboratory evidence of infection, while 19.3 per cent had colon bacilli in one c.c. amounts and 15.4 per cent in 100 c.c. amounts. As the initial infection originated in the city supply, these wells played little if any part in the original infection, but were examined because of the popular opinion that they may have been factors in the cause of subsequent or secondary cases. In nearly every instance these waters showed a higher count than samples from any portion of the public supply taken on the same date.

PRIVATE SUPPLY PHYSICAL AND CHEMICAL EXAMINATIONS.

As might be expected in dealing with wells of varying depth, construction, and environment, the variation in the quality of the water as revealed by chemical and physical examinations was very great. Fifteen chemical and physical examinations were made from various sources but neither at the time of the examination,

nor since, has it been possible to connect the quality of the water with the occurrence of primary or secondary typhoid cases. Single examinations of each source were made and it has not seemed of sufficient importance to warrant analyses of later samples. Comparisons of details are therefore omitted.

EXAMINATIONS OF BLOOD FOR WIDAL REACTION.

The Mankato authorities were fully alive to the possibility of a typhoid epidemic following the infection of the city supply, and specimens of blood were forwarded promptly. The first positive report was made on July 11. On July 13 two of the nine specimens submitted were reported as positive and upon two others a report of "suspicious" was made. During the period of the epidemic, from the date of the first examination, July 9, to October 27, 88 positive reports were made on specimens sent to the laboratory for examination. These represent 85 separate cases, forwarded by and reported to 26 physicians in 14 communities.

OTHER BACTERIOLOGICAL AND LABORATORY EXAMINATIONS.

Only one individual who as a "carrier" might have been responsible for the epidemic was found. This case was thoroughly investigated from the epidemiological point of view, and laboratory examinations of blood and feces were made, without any results, however, as far as isolation of the typhoid bacillus was concerned. Throughout the work search was made for other "carriers" who might have been responsible for the epidemic, but without avail. For the details of this phase of the work, see report of the epidemiological division, which follows. Other bacteriological examinations, such as blood cultures, were not made for the reasons already given in the introduction of this report.

THE EPIDEMIOLOGICAL DATA.

H. W. Hill.

The Mankato typhoid fever epidemic of June and July, 1908, is worthy of epidemiological note on account of the epidemiological statistics derived from its completed returns; for the results of the publicity work in circumscribing primary infections, and for

the successful reduction to one-half or one-third of the usual proportions of the primaries and secondaries, by daily inspection and supervision.

No brilliant or intricate epidemiological work was done in discovering the source of the primary infection, since pollution was easily recognized visually in the well-pit of the Broad Street well, and even without this the sudden extreme prevalence of diarrhea and dysentery uniformly distributed throughout the city water-takers could be accounted for in no other way than by contamination of the water supply. Hence the outbreak could be and was definitely diagnosed as a city water epidemic at the first glance.

On the other hand, the most careful epidemiological work was done concerning the sources and routes of the secondary infections.

The outbreak was chiefly remarkable for the very definite source of the primary infection; its sudden development; the very short time during which it was operative to any large extent; its equable distribution through the population; the proof (from dysentery and diarrhea) of the infection with the polluted water of practically the whole population; the relatively small number of primary typhoid bed cases resulting (less than 4.5 per cent of the population); the remarkably few secondaries; their derivation practically exclusively from direct immediate contact with existing cases; the abrupt cessation of the primary outbreak when the main cause was removed; and the almost absolute lack of slow continuance by carriers or convalescents in the succeeding years.

In this report, the first five sections deal with certain general epidemiological data and with the application of these data to the specific instance of the Mankato epidemic. The remaining sections give in detail certain specific features of the Mankato epidemic itself.

The effort has been made to show throughout this report both that this outbreak illustrates certain general epidemiological principles, and also that its data go to establish further certain general epidemiological principles.

Were the report written only for epidemiologists, the data itself, with the briefest explanations, would suffice.

It is hoped, however, that the interspersing with the data of the

deductions from it and of comments of various kinds will make the report more acceptable to those who, without being experts in this line, feel an interest in such work.

THE COLLECTION OF THE EPIDEMIOLOGICAL DATA.

It was early recognized that this outbreak promised unusual opportunities for the use of statistical epidemiology as applied to an average city of average composition having practically every inhabitant exposed through its water supply to a source invaded by a severe and sudden, but temporary infection.

Hence every effort was made to secure complete first-hand data from the patients by men especially assigned to that work and appreciating its importance. Cards were filled out for every case by Mr. Piper, Dr. Alley, or the writer (all as representatives of the State Board of Health), the data being secured at the bedside or from the immediate household. The information was checked and rechecked as new facts came to light. A special intensive house-to-house visitation, similar to that since recommended by Professor William T. Sedgwick for the investigation of the typhoid of Washington, D.C., was planned and carried out, special inspections of dairies, milk handlers, and others were made, and particular attention was paid to the differentiation of primaries and secondaries. The fact that these researches were made as the cases developed, not late in the disease or after recovery, gave to the data secured an unusual reliability in details. Thus not only were the usual data of the epidemiologist secured in the manner and quantity needed for the ordinary emergency epidemiological diagnosis and treatment, but this data was so collected, and so checked, revised, and confirmed as to be worthy of credence as scientific material for the basing of permanent deductions concerning such outbreaks.

Some months later the whole mass of data was gone over in statistical detail by the writer, tabulated, collated, analyzed, and interpreted. Finally, in 1911, a second detailed examination of the material was made and this report written, in the light of all the evidence collected to date.

Especial attention had been paid in the field to the possibilities

of secondary milk infections; the data on this subject were reviewed and every infected family which possessed even one cow or sold milk was checked against the milk supplies for every patient, in itself no small task.

The names and addresses of patients were checked against each other and compared. Correspondence was conducted concerning cases infected in Mankato, but taken sick elsewhere.

A long series of special outside investigations was made in the autumn of 1908 by Dr. Alley, who moved from place to place about the state as these "outside" cases were found and collected the epidemiological data from them in detail.

Hence the figures presented are unusually comprehensive and trustworthy. It is of course impossible that they should be absolutely accurate. At the same time, errors large enough to alter the deductions could hardly have escaped detection.

North Mankato, a small residential portion of Mankato lying on the other side of the Minnesota River and having no Mankato city water, being wholly supplied by small driven-wells, acted as an excellent check on the local conditions in Mankato proper, while valuable contrasts could be drawn in certain lines between the development of the main epidemic in Mankato and the development of the sub-epidemics lighted up in other places from visitors infected in Mankato.

THE STATISTICAL STUDY OF TYPHOID CASES FOR EPIDEMIOLOGICAL PURPOSES.

Too often in routine state and municipal records and even in the face of epidemics, typhoid cases are listed by the date on which the report was made by the physician. The rise and fall of outbreaks and even the date when infection first entered the bodies of the patients are based upon tables constructed from these dates.

When it is remembered that the average period of incubation is two weeks, varying from five to 21 or more days; that the period between the development of earliest symptoms and the first visit of the physician is six or seven days, varying from two to 21 days or more; that the physician is likely to arrive at a satisfactory diagnosis of typhoid fever only after two to six or eight days' observa-

tion and perhaps a Widal or other laboratory test; that the physician usually reports without hurry and often after considerable delay, if at all, it is easy enough to appreciate that, on the average, records tabulated by the dates on which the physician reports his cases indicate nothing but very crude and misleading outlines. To get the facts, every such case must be retabulated on the basis of the date of earliest symptoms of each individual case, before the true curves of incidence can be determined; moreover, if the deductions from the study of the Mankato outbreak be correct, the dates of infection cannot be properly determined by applying merely the average incubation period (14 days) to these corrected figures, but such determination requires the application of a formula (see p. 443 of this report) indicating the relative proportions of the chief lengths of incubation usually found, rather than a crude average of all.

That these are not "mere technical refinements," but very serious and important factors in the handling of typhoid fever can be shown easily.

Nothing is more common than to find typhoid outbreaks, if tabulated by the dates on which they are reported by physicians, attributed to causes first operative in the month of report, whereas on the average the time of infection was at least a month earlier. Long series of years of cases have been considered as autumnal typhoid and efforts made to explain them as such because tabulated by the dates of reports when consideration would show that these cases were really summer typhoid. Time and again outbreaks are attributed to specific occurrences on specific dates, occurring *just before* the (reported) outbreaks, the nearness of the specific occurrence in time to the reported outbreak being, as a matter of fact, *conclusive evidence that the two things were unrelated.*

Psychic factors, also, especially in epidemics, enter very largely into the dates of reporting. In the very beginning of an epidemic there is apt to be some delay, because the first few cases are diagnosed with extreme caution (if typhoid be rare in the community) or regarded as commonplace occurrences about which there is no hurry (if typhoid be not unusual in the community). Later on, as word gets about that an outbreak is developing, physicians become

more and more on the *qui vive*, diagnoses are made without the same hesitation, and, in stress of fear of impending disaster, are reported promptly. Still later, if cases come thick and fast, the existence of a catastrophe becomes an accepted matter—the feeling that the authorities have been aroused and are attending to the outbreak develops—and the physician becomes immersed in the strenuous task of caring therapeutically for a wholly abnormal number of cases. Hence promptness in reporting falls off again. "What matters a case more or less now, when there are so many? I will report tomorrow or next day—or will wait until I can report six or eight at once and make one job of it." Hence peculiar groupings of cases occur, due purely to these psychological factors; yet these groupings are often "explained" later on, as correlating with physical occurrences absolutely unrelated thereto.

The only remedy for this confusion and misapprehension lies in the systematic epidemiological investigation of every case as soon as reported, and the tabulation of the cases by *dates of infection* calculated from *carefully established dates of earliest symptoms.*

THE INTERVAL BETWEEN THE EARLIEST SYMPTOMS AND THE PHYSICIAN'S FIRST VISIT.

In typhoid fever, the date on which the patient begins to feel sick averages 14 days (but see p. 443 of this report) later than the date on which the infection was received, i.e., the date on which the typhoid bacilli entered the patient's mouth. These "earliest symptoms" are indefinite and are usually disregarded at the time of their occurrence, being remembered only if the patient later becomes definitely and unmistakably ill. The interval between these two dates is of considerable importance. It is sometimes quite short, often prolonged—and it is well known that light ambulatory ("walking") cases exist in which definite illness, sufficient to send the patient to bed or to inspire a call for the services of a physician, does not develop. Of course, illness which would be disregarded or struggled against by some individuals, sends others quickly to bed.

In Mankato, of the 405 recorded cases, 319 gave an average interval between earliest symptoms and the physician's first call of

5.6 days; i.e., the physician called on the sixth day after the development of the first symptoms. The remaining 86 cases gave no data, or indefinite data, or gave no interval of time, or an interval of one day only, between the two dates.

It may be taken as firmly established that no physician ever sees a typhoid case on the true date of earliest symptoms except by accident or under extremely unusual circumstances. So also it is very seldom that the patient is seen professionally after but one day of this condition. Hence in these 86 cases the earliest symptoms were antedated six days from the date when each case was first seen, and so appear in Table 1.

In North Mankato 18 of the total 22 cases gave definite intervals between earliest symptoms and the date first seen by a physician.

"Outside Mankato" yielded 60 cases, out of a total of 84, which gave definite intervals.

It is of considerable interest to note that while the average interval in Mankato was 5.6 days, the average outside Mankato was 7 days, and in North Mankato 7.5 days; i.e., the Mankato patient was seen on the average on the sixth day, the "outside" patient on the seventh day, the North Mankato patient on the eighth day.

The explanation of these differences is not perhaps hard to find. Mankato was inhabited by a mixed well-to-do population, all but panic stricken, thoroughly posted on what might happen and tending to look upon any indisposition as possible premonitory symptoms of typhoid fever. North Mankato was inhabited by a financially less well-to-do population, more chary of unnecessary expense and not panic stricken, because not supplied with the infected water. The outside cases were as a rule persons who were infected as the result of a short stay only in Mankato. They did not as a rule look forward to possible development of the disease, and when its full symptoms appeared did not appreciate their seriousness early. That these explanations are not far fetched may be shown by the fact that patients in general throughout Minnesota show an interval of about seven days, except in parts of Northern Minnesota, where the payment of a monthly fee secures the services of the physician. There the typhoid patient goes to

see a physician the third or fourth day of the attack, no hesitation or delay on account of expense entering into the matter.

The date of the physician's first visit under ordinary conditions is also the date on or about which the patient first goes to bed.

The logical deduction is, then, that the date of going to bed of the patient averages 21 days from the date of infection; but also that this period, being the sum of the incubation period and the prodromal period, both quite variable, is necessarily also itself variable. Thus with five days' incubation and two days of prodromes, the physician *may* be called on the seventh day after infection; with three weeks incubation and three weeks of "prodromes," the physician may not see the case until six weeks after infection. Hence the unreliability of records based on the date of the physician's first call or the date of going to bed. Of course it is also true that the physician does not always see the patient until the latter has been in bed one, two, or even more days; and the physician, called to see a patient still up, may not at once recognize the disease, and may see no immediate reason for sending him to bed. For all these reasons the *date* of *earliest symptoms*, checked by the date of the physician's first call and the date of going to bed, is the best date to use for calculations.

Interval Between the Date of Earliest Symptoms and the Physician's First Visit.

North Mankato (18 cases gave data).—Average interval = 7.5 days, i.e., the physician called on the average on the eighth day.

Number of days in interval,	2,	3,	4,	5,	6,	7,	8,	9,	10,	11,	12,	13,	14,	15,	16,	17,
Number of cases showing intervals,	2,	5,	3,	2,	1,	1,	0,	0,	0,	1,	0,	0,	0,	0,	0,	0,
Number of days in interval,	18,	22,	31													
Number of cases showing interval,	1,	1,	1.													

It must be noted, however, that over half the cases were seen before the fifth day.

Mankato cases (over 300 cases gave data, nearly all here listed).—Average 5.6 days, i.e., the physician was called on the average about the sixth day.

Number of days in interval,	2,	3,	4,	5,	6,	7,	8,	9,	10,	11,	19,	21,	23
Number of cases showing interval,	55,	57,	53,	42,	18,	55,	12,	12,	4,	3,	2,	2,	1.

It must be noted that over half the cases were seen before the fifth day. (Some scattering intervals were not given.)

Outside cases (58 cases gave data).—The average was seven days.

Number of days in interval,	2,	3,	4,	5,	6,	7,	8,	9,	10,	11,	12,	13,	14,	20
Number of cases showing interval,	8,	3,	5,	10,	5,	7,	2,	3,	5,	1,	4,	1,	3,	1.

It must be noted that half the cases were seen before the seventh day.

These tables show that publicity and the fear of typhoid in Mankato drove the people to consult a physician early—soon after symptoms began. Outside Mankato the average conditions existed and a physician was not called until the symptoms became more severe.

INCUBATION PERIODS.

The incubation period of typhoid fever is the interval between the date of infection and the date of development of the earliest symptoms. As outlined in considering the period between the latter date and the date on which the case is first seen by a physician, it is often difficult to fix accurately the date of earliest symptoms. Hence, although the incubation period is generally considered as averaging 14 days, some epidemiologists prefer to fix the date of infection as 21 days before the first visit of the physician, or the date of going to bed, rather than at 14 days before the date of earliest symptoms. The writer used a new method (see p. 443 of this report) based on these incubation periods and applied to the question of the continuity of the infection.

The fact that a number of cases developed typhoid fever at various points outside of Mankato after receiving infection in Mankato during visits limited to one, two, or a few days, gave the opportunity for determining their incubation periods with some degree of accuracy.

Those whose stay in Mankato was for but one day naturally give the most definite information. Of such there were 12 males and nine females, giving an average of $13\frac{17}{21}$ days, without discrimination of age or sex. One of these gave a period of 29 days, so unusually long as to appreciably affect the average. Omitting this one, the average is $13\frac{1}{20}$ days. Hence the earliest symptoms developed on

the average on the 14th day, but with wide variations in individual cases.

Those whose stay in Mankato exceeded one day were used for supplementary information, by calculating the date of infection as the middle of the visit. Of such "indefinite" cases there were six males and four females, giving an average of $15\frac{1}{2}$ days. If, however, we assume that each of this group received infection on the first day of the visit instead of the middle of the visit, the incubation periods for this group would average $13\frac{6}{10}$ days, thus giving the average date of earliest symptoms as the 14th day.

If the "definite" incubation periods for males and for females be averaged separately, the average period for females ($15\frac{1}{9}$) is found to be slightly longer than that for males ($12\frac{10}{12}$). However, if the two extreme incubation periods be omitted (the shortest five days, from the male list, the longest 29 days, from the female list) it is found that both lists yield the average date of earliest symptoms as the 14th day.

The "indefinite" periods, however, when thus arranged, indicate a longer incubation period for females than for males, whether the date of infection be assumed as occurring in the middle of the visit or on the first day of the visit. It would seem, then, that females may on the average develop symptoms of typhoid fever a day or two later than males. The individual cases are not, however, numerous enough to permit any very conclusive deductions. To offer an explanation of what may not be a constant feature would be premature.

FEMALES.

Number	Age	Period from Date of Infection to Date of Physician's First Call	Incubation Period
18	24	11	5
10	19	24	10
72	16	13	11
74	15	25	15
60	20	13–21	7–15
9	20	19–22	16–19
11	15	25	18
56	27	25	18
76	26	21	16
79	17	34	29
8	21	34	14
61	11	25–26	19–20
17	12	30–33	22–25

MALES.

Number	Age	Period from Date of Infection to Date of Physician's First Call	Incubation Period
7	15	19–20	17–18
81	18	26	17
55	20	19	7
30	21	11	5
15	21	20	15
31	22	17	12
82	24	19–25	16–22
63	26	31	20
5	28	16–22	10–16
20	28	28	18
71	30	16	8
80	30	24	17
83	32	15–18	9–12
34	34	20	10
77	44	14–15	6–9
59	44	12	8
4	14	29	17
1	39	19–25	12–18

SOURCE OF THE TYPHOID INFECTION OF THE MANKATO CITY WATER.

Two distinct outbreaks of two different diseases existed in Mankato as the result of Mankato sewage entering the supply on or about June 25, 1908.

The first, most extensive, and that which earliest caught widespread public attention, was termed for convenience the initial diarrhea. The second, a smaller and later outbreak, was a classical epidemic of typhoid fever.

The initial diarrhea involved adults and children of both sexes and all ages to the number of 4,000 to 6,000 cases, i.e., fully half of the drinkers of city water suffered. Eleven horses are stated on the authority of a prominent local veterinarian to have shown similar symptoms. Occurring principally as the result of the pollution dating about June 25, these cases seemed to yield probable incubation periods of 0–4 or 5 days with exceptionally longer periods which could not be fixed at all absolutely. Many of those who visited Mankato for but a day during this period and drank the city water contracted diarrhea, but the date on which the diarrhea occurred as compared with the date of drinking the water is not recorded in any such case.

In one instance death occurred indirectly attributable to this diarrhea. A female aged about 38 arrived in Mankato during the diarrheal outbreak, June 27, drank the water and at once developed

very violent vomiting and purging, dying the evening of the day on which she arrived. The actual cause of death was cerebral hemorrhage, induced by the exhaustion and strain of the attack. This was the only death attributable to this disease in Mankato.

The disease clinically varied very much—from mere passing looseness with or without slight pain or general ill-feeling to fulminating attacks quite like ptomaine poisoning in their violence and the extent of the collapse. Some cases lasted a day or two, others a week or two.

The persons who suffered the initial diarrhea contracted typhoid fever in greater proportion than did those who escaped the initial diarrhea.

Experiences in St. Peter in July, 1908, and in 1909, at the University Agricultural School in 1907, and in Hibbing in 1910 show that this diarrhea may occur without a subsequent typhoid fever. These four outbreaks of diarrhea furnished respectively 300–400 cases, 300–400 cases, 600 cases, 1,600–2,000 cases, in each instance involving a very large proportion of the total water-takers. The clinical symptoms and especially the wide variation in severity and in the length of the attack were similar in all instances. All four of these outbreaks were due to water-infection; no one of the four resulted in even a single case of typhoid fever. In one instance the infection was due to the admission to the artesian water supply of ordinary domestic sewage. In one instance it was due to pollution by laborers working in the deep shafts from which the water supply was derived. In the other two instances it was due to infection of artesian water with river water, at that time in flood, the flood water bearing the sweepings of the river valley above the point of infection and the sewage of at least one city, greatly diluted, however.

Other outbreaks of a similar character, some associated with typhoid fever, some not so associated, have been encountered in Minnesota and traced conclusively to fly-carriage of human discharges from outdoor toilets or similar exposed deposits of human feces and urine, possibly to similar carriage of garbage(?) or of horse manure(?).

Hence we know absolutely from Minnesota studies that the admission of human discharges to human food or drink, may

produce, according to the amount of the discharges, or their character, or both, first, no effects; second, effects on the alimentary tract, non-typhoidal but extremely severe or extremely mild or intermediate in character, quickly passing off; third, typhoid fever. Also that *typhoid-infected* discharges may produce conditions given under 1 or 2 or 3, or 2 followed later by 3, while discharges not so infected can produce only results as given under 1 or 2.

We have every reason to believe that, while we do not know just why or when or how the intestinal disturbances above described, other than typhoid, are precipitated, we do know that typhoid fever cannot occur unless the discharges taken with the food or drink contain the typhoid bacillus. Thus we are safe in saying that in the outbreaks at St. Peter, the Agricultural School, and Hibbing, above referred to, typhoid bacilli were not present in the material which infected the supplies in quantities sufficient to precipitate disease. On the other hand, where typhoid fever occurs we know that human discharges containing typhoid bacilli were involved, for these are the only known sources of typhoid bacilli, and moreover their presence fully accounts for all instances. Hence, the development of typhoid fever at Mankato from the city water demonstrates, not only the pollution of that supply with human discharges, but also the pollution of that supply with *typhoid-infected* human discharges. The initial diarrhea is fully and completely explained by the admission of human discharges to the supply, whether these discharges contained typhoid discharges or not. The typhoid outbreak makes it certain that the discharges contained typhoid bacilli also.

Whence came these typhoid bacilli? The most careful search of the records of hospitals and physicians' records showed no typhoid fever cases contributing to the sewage for many months previous to the outbreak, with the exception of one case, infected elsewhere and sick at home during the preceding December, January, and February. On recovery this patient continued work in Mankato.

It is not to be believed that the discharges of this patient so infected the sewage in February that at the end of June enough bacilli remained to precipitate the Mankato epidemic.

The possibility that this patient might have become a chronic

carrier, voiding typhoid bacilli, and so more or less continuously infecting the sewers up to the end of June, must be admitted.

Examinations of this patient's feces and urine were made by the laboratories of the State Board of Health to elucidate this point, but bacilli were not found, nor does any history exist then or since, of immediate associates of this patient contracting the disease from her—as would probably have been true were this patient a chronic carrier.

Hence we are forced to conclude that the typhoid infection unquestionably present in the city water was derived from the discharges of persons unknown, probably transients, early cases, convalescents, walking cases, or even carriers, among the general population in Mankato at this period.

Transients are common in Mankato—traveling men, business men of all descriptions, visitors. At the end of June, the Normal School was drawing widely from all over Minnesota, and business colleges, etc., were running. A ministers' convention met June 24–30. A circus was present June 25. Hence Mankato had every opportunity to entertain many infected persons, and, owing to the fairly complete sewerage systems, the discharges from these had every opportunity of mingling with the general sewage. When the latter entered the water supply the infected discharges entered with it.

DATE OF FIRST INFECTION OF MAINS AS DERIVED FROM THE OCCURRENCE OF THE INITIAL DIARRHEA.

The initial diarrhea, in persons later developing typhoid, so far as definitely dated, occurred on June 25 in 27 cases, June 26 in seven cases, June 27 in two cases, July 4 in one case. The intensive investigation discovered the initial diarrhea in persons not developing typhoid as originating June 25 in 13, June 26 in 34, June 27 in two, July 1 in two, July 4 in five, "June" in 13, "July" in one case. These included Mankato and North Mankato people. Among the typhoid cases which developed outside Mankato, some are recorded as being in Mankato but one day; others two, three, or four days only. It is significant that no outside typhoid case developed as the result of any visit terminated previous to June 25.

Of those in Mankato on June 25 only, who later had typhoid, 11 developed diarrhea; on June 27 only, one developed diarrhea. Six cases present in Mankato from two to eight days, beginning June 24, developed diarrhea. A single case, attributed to drinking Mankato water on July 18 only, showed some initial diarrhea. In no other case is diarrhea attributed to drinking the water later than July 4.

Thus a total of 51 cases of diarrhea definitely correlate with infected water as early as June 25, but not one case earlier. The incubation period of this diarrhea is, we have reason to believe, from a few hours to several days. Hence there is conclusive evidence of infection of the water on June 25, but no evidence of any earlier infection. How long infection of the mains lasted, capable of producing this diarrhea, cannot be determined definitely. It seems evident that the bulk of this infection occurred in June; that a small proportion occurred in early July; one case possibly as late as July 18.

A STUDY OF INCUBATION PERIODS TO DETERMINE CONTINUITY OF TYPHOID INFECTION.

From the admittedly meager data on incubation periods (21 definite and 10 indefinite) we may tentatively calculate that taking the intermediate figure in each "indefinite" as the true period for that indefinite, we have 31 incubation periods which may be arranged thus by date of earliest symptoms.

Days after infection,	5th,	7th,	8th,	9th,	10th,	11th,	12th,	13th,	14th,	15th,	16th,
Cases,	2,	1,	2,	1,	2,	3,	1,	1,	1,	3,	1,
Days after infection,	17th,	18th,	19th,	20th,	24th,	29th.					
Cases,	3,	5,	1,	2,	1,	1.					

Hence of 31 people infected on a given date, there would be three who showed symptoms within one week later, 11 within another week, 15 within the third week, one in the fourth, and one in the fifth week.

Without attaching too much importance to the actual relative proportions in which the different incubation periods occurred in this outbreak as representative of the usual proportions, it is nevertheless very curious and interesting to note that if we assume

a violent infection on June 25, continuing but diminishing, so that but two-thirds as many were infected by the end of the first week, and but one-sixth as many by the end of the second week, we obtain the following proportions:

	1st Week	2d Week	3d Week	4th Week	5th Week	6th Week	7th Week
Proportion for 31 people	3	11	15	1	1	0	0
Proportion for 186 people infected June 25	18	66	90	6	6	0	0
Proportion for 124 people infected by end of first week thereafter	..	12	44	60	4	4	0
Proportion for 31 people infected by end of second week thereafter	..	..	3	11	15	1	1
Theoretical results by dates of earliest symptoms 341	18	78	137	77	25	5	1
Actual returns of earliest symptoms 349	13	75	151	75	21	12	2

This rather startling parallelism between the purely theoretical and the actual returns is somewhat further enhanced if in the fifth week two cases returned as possibly secondary be added to the actual returns. So far, then, it would appear that the supply was indubitably infected June 25 to July 9 and that an infection between these dates would account for nearly all the cases which showed symptoms to July 30.

Two factors would account for a decided diminution in the number infected at the end of the first week—notification that the water was infected, with directions to boil it, first given late in June, and gradual dilution of the infection by inflowing pure artesian water; probably also the natural dying out of the bacilli. All these factors continued to operate during the second week and with increasing effect; but some persons continued to drink the raw water to the end of the third week after infection, thus doubtless extending the primaries. The typhoid infection of the mains seems to have continued, on other evidence, at least as late as the 18th and perhaps the 25th, as on these dates visitors (three on the 18th, one on the 25th) to Mankato for one day only became infected. By this time residents almost without exception had abandoned the use of raw water, many boiling even well-water wholly unconnected with the city supply. In certain instances, however, Mankato residents not exposed to secondary infection, so far as careful investigation showed, admitted drinking city water

late in July and contracted the disease, but these cases might have been untraced secondaries.

It may be considered that 30 days (June 25 to July 25) or even 23 days (June 25 to July 18) is a rather long period for typhoid bacilli to remain in water in a condition capable of producing typhoid fever, especially in view of the constant dilution of the pollution and the washing out of the mains due to constant influx into the water main system of artesian water, and to extensive flushing done specifically for these purposes.

The usual period of persistence of infection in a surface water exposed to sun and air and not changed by inflow and outflow is currently held to be not longer than two weeks. Whether or not this period has ever been determined *in view of the proportionate distribution of varying incubation periods* as given above, is unknown to the writer. Nevertheless, there is evidence first obtained here in 1907, from the records of the Breckenridge typhoid, that the shutting out of sun, light, and air by ice in winter on the Otter Tail River conduced to greater longevity or virulence, or both, of the typhoid bacilli. This was later noticed by Rüdiger regarding the Red Lake River and still later worked out experimentally by him, with quantitative experiments on the actual reduction in longevity of typhoid bacilli in Red Lake River water in summer as compared with winter.

Artesian water flowing directly into mains would be, if infected, somewhat in the same condition as regards light and air as surface water in winter flowing under ice. Hence it would be proper to expect a greater longevity of infection in mains under the Mankato conditions than in an open surface water.

Unfortunately for all concerned, it cannot be demonstrated from engineering, meteorological, or other data that the mains received infection only on June 25, although there is also no proof of typhoid pollution entering the supply later than that date. Indeed it is not improbable, although not now to be proved, that the mains received new infection for some time—how long cannot now even be guessed. Hence there is no way of determining with any degree of certainty that typhoid bacilli survived in the mains for a greater period than they would have survived in surface water. On the

hypothesis that a single infection only occurred (on June 25) it would appear conclusively that they survived for at least two full weeks; almost conclusively, for 23 days; on some evidence, for 30 days.

Relation of an Attack of the Initial Diarrhea to the Development or Non-development of Typhoid Fever in the Same Persons

Initial Diarrhea.	Typhoid.	
+	+	= 206 persons
−	+	= 53 persons
+	−	= 253 persons
−	−	= 144 persons

Total with initial diarrhea = 459 (typhoid followed in 206).
Total without initial diarrhea = 197 (typhoid followed in 53).

Hence nearly half of those with initial diarrhea had typhoid, while only a little over one-fourth of those who did not have the initial diarrhea suffered later from typhoid fever.

It would seem, then, that there was no evidence, as has been supposed, that the initial diarrhea purged the system of the typhoid bacilli and so reduced the tendency to typhoid.

The question of how many persons suffered in Mankato from the initial diarrhea may be answered approximately by these figures. Thus, of 397 non-typhoid persons showing data, 253 suffered the initial diarrhea and 144 did not. If the 10,000 water-takers in Mankato suffered in approximately the same proportions as those investigated there were about 6,000 who had the initial attack of diarrhea. This figure is not exaggerated, as inquiry showed that the majority of all persons questioned gave a history of an attack. Among these 6,000, then, occurred 206 cases of typhoid. We know that of the 4,000 remaining citizens only 53 had typhoid. (Data on the remaining 96 primary typhoids is indefinite on this point.)

Hence those who escaped the initial diarrhea constituted two-fifths of the population but they supplied only about one-fifth of the total cases of typhoid.

TOTAL CASES AND SUBDIVISIONS.

The Mankato typhoid infection resulted in the development of 511 typhoid bed cases of which we have definite records. Owing to the minute watchfulness of the local Health Department and the State Board of Health representatives, but few if any bed cases escaped record.

The recorded cases include (1) primary cases (440); i.e., infected directly by drinking water containing typhoid bacilli in Mankato. Certain of these cases (20) occurred among residents in North Mankato, certain others (66), among those temporarily in Mankato as visitors or on business. The majority (354) naturally occurred among permanent residents of Mankato.

(2) Secondary cases; i.e., infected, not by taking typhoid bacilli into their systems in the drinking water, but by taking them directly from existing sick persons—the discharges of the latter reaching the mouths of the secondary cases on fingers (their own or intermediary), or in food infected by the sick, by intermediaries, by soiled laundry, or by flies. This latter group is important because it consists of cases which were not an *essential* part of the general outbreak—since they need not have occurred, notwithstanding the primary outbreak, if the theoretically correct sanitary care and sanitary nursing of the patients had been carried out in minute detail. As it was, the unusual sanitary care taken in this outbreak in the nursing of the sick under the constant surveillance of those in charge of the epidemic, reduced the secondary cases much below the usual ratio of such cases to primary cases.

These secondary cases are likewise divided into those which occurred in North Mankato (two), those occurring outside (18), i.e., in communities to which went some of the primary cases infected in Mankato, and those occurring in Mankato itself (51). This division is useful for a number of reasons and particularly brings out the point that the secondaries were more abundant among outside cases than in Mankato and North Mankato, where the publicity, warnings, and supervising visits continuously given emphasized the danger and pointed insistently to the preventive measures.

TABLE 1.

TYPHOID CASES INFECTED DIRECTLY OR INDIRECTLY BY MANKATO WATER.

	North Mankato			Outside			Mankato		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Primary	12	8	20	26	40	66	161	193	354
Secondary	0	2	2	7	11	18	15	36	51
Total	12	10	22	33	51	84	176	229	405

Total primaries, 440; total secondaries, 71; grand total, 511.

This table represents the most accurate classification that could be made from the data at hand. It is not always possible to determine whether a case is primary or secondary, *so long as* the primary source evidently continues to operate. Moreover, it is not always possible to determine just when the primary cause ceases to act. Hence there is often an interval when a double uncertainty must exist, i.e., during that period when it is not clear whether or not infection from the primary source has ceased. Thereafter, of course, every case occurring must be secondary.

These periods were fairly well defined in the Mankato epidemic by the epidemiological evidence, i.e., that relating to the cases themselves, as distinct from meteorological, engineering, or laboratory data concerning the environmental conditions.

Hence of 317 families involved, 32, or about 10 per cent, yielded secondaries. Of the 285 families giving primary cases only, 41 families, or about 14 per cent, yielded more than one primary case, or a total of 91 cases in 41 families.

Of the 32 families yielding secondaries, 16 families showed one case only (but that one secondary) while 16 families showed two or more cases, one or more being secondaries. In one of these families there were two secondaries, without any primary within the family. (The first secondary received infection from work in a laundry, handling infected clothing; the second received infection from this first.) The 15 remaining families showed one or more primaries followed by one or more secondaries, a total of 19 primaries and 29 secondaries.

In at least four of the 16 single-case (*secondary*) families, possible walking typhoids in the family existed. These walking cases, if they existed, would furnish the cases primary to the secondary

TABLE 2.

CASES SHOWING DISTRIBUTION IN FAMILIES.

	Families with Primary Cases Only	Cases in These Families	Families with Primary and Secondary Cases	Primary Cases in These	Secondary Cases in These	Families with Secondary Cases Only	Cases in These	Total Families	Total Cases
1-case families...	244	244	0	0	0	16	16	260	260
2-case families...	32	64	5	5	5	1	2	38	76
3-case families...	9	27	6	9	9	0	0	15	45
4-case families...	0	0	2	3	5	0	0	2	8
5-case families...	0	0	1	1	4	0	0	1	5
7-case families...	0	0	1	1	6	0	0	1	7
Total.........	285	335	15	19	29	17	18	317	401
Hospitals yielded four secondary cases									4
Total									405

cases—which later however, were alone recognized. In a number of instances (six) certain cases listed as outside Mankato, because their illness occurred elsewhere than in Mankato, were connected with families in town where they were present as visitors or domestics or boarders, etc. These cases, had they been sick in Mankato, would have slightly increased the number of multiple-case families in Mankato.

Nurses infected while nursing typhoids in private families were counted as members of the families concerned.

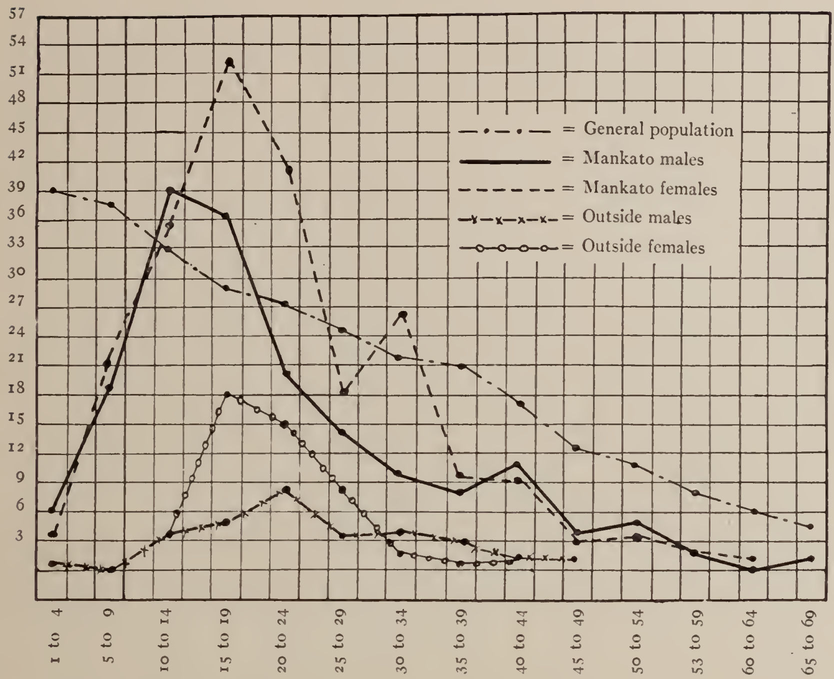
GENERAL AGE AND SEX INCIDENCE AS COMPARED WITH GENERAL AGE AND SEX COMPOSITION OF THE POPULATION.

A map of Mankato, showing both the city water mains and the primary cases, indicated a practically uniform and homogeneous distribution correlating with the city water distribution. Some families and small neighborhoods, using largely private well-water even in the city districts, escaped. On the other hand, some persons outside the city water district, notably in North Mankato, succumbed, but in all instances the primary cases from these outside districts were found to use city water while at work or on trips "down town." These exceptions were rather rare, and the North Mankato cases are separately classified.

Hence it was true that this outbreak affected the Mankato population pretty uniformly, and that the cases were drawn, fairly proportionately to the composition of the population, from every class of that population, whatever the basis of classification.

A comparison of the age incidence of the disease in Mankato with the age composition of the general population as given in the census report of 1900 is therefore of interest, although no very minute deductions are proper. It is worth noting that, although the average age distribution of the population decreases pretty uniformly as the age increases, the most abundant ages (young children) supply the smallest number of cases (children under 10 form about 27 per cent of the Minnesota population, but yielded only 12 per cent of the cases). This comparative immunity of

Chart 1.



Total cases occurring in Mankato (excluding North Mankato) and outside, by age and sex.

Males, continuous line—Females, interrupted line—Age distribution of general population, dotted line.

Mankato males listed, 175. Females listed, 227. Total, 402. (Three cases—one male, two females—age not given.)

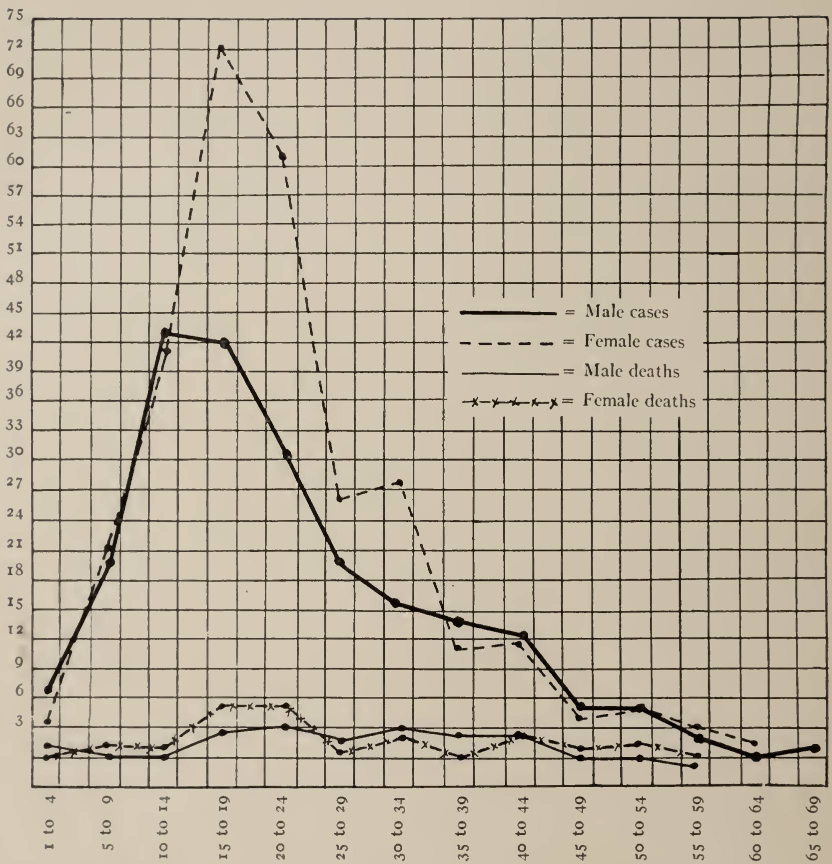
Outside males listed, 31. Females listed, 51. Total, 82. (Two cases—males—ages not given.)

In Mankato, female patients exceeded male patients at ages 15 to 24, and again at 30 to 34. Otherwise the parallelism is quite remarkable.

While the excess is partly accounted for by the excess of female secondaries (due to greater exposure in nursing, etc.) over male secondaries, it is in part due to an excess of female primaries, probably due to a variety of sociological reasons which gave to the general population of Mankato an excess female population. As a well-to-do manufacturing, business, and scholastic center, females in many capacities were engaged as office girls, clerks, stenographers, students, servants, etc.

The excess of outside females over outside males is wholly accounted for by the excess females among Normal students and teachers, visitors and secondaries.

Chart 2.



Total cases due to Mankato infection (Mankato, North Mankato and outside) by age and sex.
Males, continuous line—Females, interrupted line

Males listed, 218. Females listed, 288. Total, 506. (Five cases—three males, two females—ages not given.

The excess of females from 15 to 34 is due in part to an excess of females exposed to the infection, as visitors, Normal and other school students, domestics, etc., but also in part to the greater exposure of females to secondary infection when acting as nurses to primary cases.

The statement that typhoid fever selects males rather than females, where exposure is equal, was evidently wholly untrue in this outbreak, the sexes being infected primarily in proportion to their relative abundance.

children ceases in the age-group 10–14 years, and thereafter the incidence in the males runs roughly parallel with their numerical proportions in the population. The lines indicating incidence in females, however, do not become even approximately parallel until the group 35–39 is reached; thereafter the curves of male and female cases are almost identical.

A partial reduction in the discrepancy between male and female incidence is seen at the female age-group 25–29, but a sharp female rise occurs in the next age-group.

The marked disparity between the numbers of males and females attacked is seen at ages 15–34, in which it reaches an excess of 78 females over males (109 males, 187 females).

In North Mankato, the disparity is small; indeed there is one male in excess for the whole age-group. At 15–24 females are three in excess; from 25–34 males are four in excess. The absolute figures are small, but seem to correlate with a tendency for the younger females (all, except one secondary, employed in Mankato) to work in Mankato; the older women remaining at home and escaping infection. The older men on the other hand (all the men affected were employed in Mankato) were at work away from home, and naturally in Mankato, where the call for their labor was greatest.

In those counted as "outside Mankato," the total disparity is 22 females in excess, 20 of the excess being between 15–24.

The female secondaries, six (excess three), are double the male, three. The normal school furnished four teachers and eight students, all females (excess 12). The business college supplied an excess of one female. Among the visitors, there were males 16 and females 18 (excess two). Five female servants against one outside male clerk complete the list of help claiming their homes outside. It is evident, then, that the excess in the females present in Mankato from outside homes at the time of the outbreak came from the classes in the general population which were in excess in females, and from secondaries—women being naturally with the sick more than men and hence more likely to be infected. (Four additional female normal students are recorded in the Mankato lists, although properly, perhaps, belonging here. This would add

to the excess females outside without disturbing the above deductions—rather strengthening them.)

Mankato itself shows an excess of 57 females—or 53, if the four female normal students just mentioned be omitted. An actress and a servant, both from outside, might also be subtracted.

The female secondaries were 20 (excess 17) as against three males. This is due undoubtedly to the fact that the males of these ages avoid the sick, while the females gravitate to them as nurses, etc. But, discounting for outside and secondary females, the proportion still remains (nearly) four females to three males infected. The census report of 1900 gives females slightly in excess of males, without distinction, unfortunately, as to particular age-groups. General information concerning Mankato seems to indicate that females exceeded males decidedly in the general population at the date of the outbreak, but no definite figures are available.

The following conclusions seem justified:

1. That the apparent excess attack of typhoid fever at the ages 10–29 is due simply to the greater abundance of these ages in the average population. Hence that typhoid fever does not select these ages out of proportion to other ages.

2. That the relative escape of children under 10 from typhoid fever is established and is more marked than is usually supposed, when the abundance of these ages in the average population is considered. Hence children, when equally exposed (apparently) with adults, show a very decided but gradually diminishing escape (whatever the reason for that escape may be) between birth and 10 years of age.

3. That age and sex succumbs to typhoid fever approximately in direct ratio to the exposure to infection; and that equal numbers of males and females of a given age, equally exposed, would succumb in about the same proportions, regardless of age, except as concerns the groups below 10 years, and perhaps above 70.

DISCUSSION OF ALLEGED SUSCEPTIBILITY OR IMMUNITY FACTORS IN THE INCIDENCE OF TYPHOID FEVER.

At first glance, the fact that a population of approximately 10,000 city water-takers in Mankato, at least 6,000 of whom drank

the polluted water (as shown by the development of 6,000 cases of the initial diarrhea), developed only about 500 bed cases of typhoid fever, might be taken to indicate a widespread resistance to typhoid fever among the general population. That such a relative resistance (whatever its nature) exists among children under 10 has been shown. Children of these ages form approximately 27–30 per cent of the average Minnesota population but contributed only 12 per cent of the cases in Mankato. It remains, then, to account for the fact that the remaining 70 per cent (7,000 persons) showed less than 500 bed cases. (We may admit an additional equal number of walking cases and still ask a similar question.)

Let it be remembered to begin with that Mankato had been free from typhoid fever for many years, so that acquired immunity to typhoid fever among its citizens was rare. Also that the alleged effect of the initial diarrhea in "flushing out" the intestine in such a manner as to drive out the typhoid bacilli ingested at the same time, is disproved by a consideration of the actual figures (see p. 446).

Admitting, then, about 1,000 cases (bed and walking) of typhoid fever from a population of 10,000 persons, the escape of the remaining 9,000 persons might be explained on the following hypotheses:

1. That only the 1,000 persons who succumbed did actually at any time take typhoid bacilli into their mouths.

2. That a much larger number took typhoid bacilli into their mouths, but that in only 1,000 of these did the bacilli survive the ordinary defenses of the body encountered during the journey from the mouth to a point where they might so establish themselves as to produce symptoms.

3. That practically the whole population took typhoid bacilli into their mouths (practically the whole population took the *polluted water* into their mouths); that in a large proportion of cases these typhoid bacilli survived the ordinary defenses of the body encountered *en route* to a *point d'appui* (this was evidently true of the *dysentery-causing* agents in at least 6,000 persons): but that in only 1,000 persons did the typhoid bacilli, after reaching this *point d'appui*, succeed in so establishing themselves as to become disturbing factors in the body economy.

In brief it might be urged that in about 9,000 of the 10,000 persons exposed a specific immunity against typhoid fever existed; and that, since this immunity could not have been acquired immunity in any large proportion of the persons, natural immunity to typhoid fever must be very common in the average population.

Concerning (1), it may easily be shown that practically the whole population drank the polluted water. The equable distribution of the cases, both of the initial diarrhea and of the typhoid fever, throughout the city, showed that the physical distribution of both in space were coextensive. A consideration of incubation periods, with dates of earliest symptoms, shows that the typhoid fever infection was coextensive with, even exceeded, the initial diarrhea, in its distribution in time.

On the other hand, it is inconceivable that the typhoid discharges present in the sewage which infected the water supply could have been quantitatively more than a very small fraction of the total discharges present in the sewage. We may conceive of so equable an admixture of the sewage with the water that abundant bacilli, say *B. coli*, would be almost uniformly suspended in every gallon, even every glassful of the water, so that practically everyone who withdrew any of the supply for drinking purposes, withdrew a considerable number of colon bacilli at the same time. But it is difficult to conceive of an equally uniform suspension in the water of the very much smaller number of typhoid bacilli. It seems likely that these bacilli would exist in suspensions more dense at certain times and in certain places than at other times or other places; in brief, that the typhoid bacilli, if visualized in the mains, would show not a uniform homogeneous density of suspension, but a more or less irregular alternation of "condensations and rarefactions." The chances that any given water-drinker, taking from the mains his daily gallon or less for drinking purposes, should draw his quota from a "condensation" rather than from a "rarefaction," would be small as compared with his chances of drawing out a condensation of *B. coli*—especially when it is remembered that the *average* density of the colon bacilli suspension would be as great or greater than that of the most concentrated typhoid "condensation."

Admitting, as we must, that *dosage* is a large factor in efficient typhoid infection, the water-taker would have many chances of imbibing only a subminimal dose of typhoid to each one that he had of imbibing a subminimal dose of say *B. coli* (*B. coli* is used merely as a *type* of the sewage germs which we presume caused the initial diarrhea).

Concerning (2), we must admit that the gauntlet which even a large dose of typhoid bacilli must run in the body between the mouth and the *point d'appui*, may result, when the body forces are at a high level of efficiency, in the destruction or "loss" of the bacilli *en route*. That a small dose should be more easily and hence on the average more often "lost," seems evident.

Concerning (3) we may admit specific immunity as operating in some cases to prevent the production of symptoms in certain persons, even after the typhoid bacilli had reached the *point d'appui*. In view of the above discussion of (1) and (2) it seems unlikely that this specific immunity, even if very common among the population, could have had in this outbreak many opportunities for going into action. That it could have been a large factor in the escape of the population from the disease seems therefore highly improbable.

Finally, on consideration of the secondary cases, it was found that nearly all were probably exposed to the original infection; it is true that somewhat less than one-half showed no initial diarrhea, while concerning a large number, no evidence on this point exists. Eight secondary cases, however, suffered from the initial diarrhea, i.e., they unquestionably drank the polluted water. Inadequate as this evidence is, we may be assured that in these eight cases at least the escape from the original typhoid infection was not due to permanent specific immunity—else they would not have succumbed to secondary infection a month or two later. If such immunity was not the explanation for the original escape of these eight secondaries (and probably not for any of the other secondaries) the explanation for their escape must be found under (1) or (2) as discussed above; and there seems to be no adequate reason why the same explanation will not apply to the other 9,000 who escaped (except as regards the children under 10).

On the apparent evidence offered by the fact that the large majority of persons artificially inoculated with typhoid bacilli for protective purposes react to such inoculation, natural immunity to typhoid fever must be rather rare.

Hence on all these grounds, it would seem that the incidence of typhoid fever in Mankato—or in any community—is a matter of *efficient dosage* rather than of specific susceptibility or immunity; and that non-specific, almost accidental, protective factors acting between the entry to the mouth and the reaching of the *point d'appui*, more often come into play than does specific natural immunity.

POSSIBLE INFECTIONS THROUGH MILK.

The milk supply of every patient was carefully investigated in order to determine whether or not milk might be a factor at any time in this outbreak. The great variety of the milk supplies of the cases, especially of the early cases, and the uniform and homogeneous distribution of the cases wherever the Mankato city supply was used as drinking water, quickly and conclusively excluded milk as responsible for the main outbreak. The question still remained whether or not milk was a factor, during the secondary period (1) through the infection of milk by early cases among milk handlers; or (2) through the use of city water to wash milk cans or even to dilute milk; or (3) by the return to new families milk-filled, of improperly cleaned milk bottles, collected from infected families.

Not only direct inquiry at the houses of patients, but inspections of milk supplies and examinations of "help" were made, the latter in order to detect possible sick or convalescents handling the milk. Further, the data obtained were most exhaustively tabulated and cross-checked: the patient sorted out by milk supply, the milk supplies checked against the cases among their customers, dates of infection of patients were compared with dates of infection of milkmen when these showed histories of attacks.

A priori, anyone familiar with the usual milk supply of the usual Minnesota community would hardly expect any great outbreak to occur from milk, for large milk routes hardly exist here as yet, and

although customers of any one milk supply may run into tens of families, they rarely reach to hundreds. Nor are these milk dealers likely to handle mixed milk from many sources. They deal pretty directly with the original cow owner as a rule. Hence the chances for great outbreaks such as are on record in eastern cities, do not usually exist. On the other hand, the carriage by milk of typhoid fever (and other infections) on a small scale is constantly occurring.

The 405 patients in Mankato were distributed to different milk supplies thus:

Milk supply No. 1	=50 patients	Milk supply No. 8	= 6 patients
" " " 2	=32 "	Own cows	= 31 "
" " " 3	=19 "	Neighbors' cows	=172 "
" " " 4	=16 "	Condensed milk	= 1 "
" " " 5	=16 "	Not given	= 40 "
" " " 6	=15 "		
" " " 7	=13 "	Patients, total	=405

(Six patients received milk from two sources each, hence the above total is 411 representing 405 patients.)

The 405 patients had over 100 separate sources of milk supply. (Some "neighbors" and small dealers supplied two or more patients each.)

The 22 patients in North Mankato showed:

Mankato milk dealers	= 4 patients	Neighbors' cows	=14 patients
Own cows	= 4 "		

The 84 outside patients showed:

Mankato dealers	=16 patients	Neighbors' cows	= 7 patients
Own cows	=48 "	Not given	=13 "

The "outside" cases receiving milk from Mankato dealers were those who lived in Mankato for some little time, although sick at home elsewhere; the very large proportion of "own cows" is due to the fact that many were farmers. It must be admitted that the outside cases which were only in Mankato a very short time could seldom state the source of their milk supplies while in Mankato. But, so far as possible, their known residences in Mankato were checked up against the milkmen known to supply those residences.

The search for secondary milk outbreaks failed except to reveal possible sources as follows—of those supplying milk in Mankato to other people, all dealing on a very small scale, a number had typhoid themselves or in their families; while one large firm had one milker sick with typhoid; and another large firm employed a boy to cap bottles whose sister had typhoid, and he himself contracted it later.

Taking into account customers of these who contracted typhoid in from one to three weeks after the date of onset in the possible infector, we find six infected small milk dealers having one case each develop among their customers within this period; but these cases might equally well have developed from the city water. We find two cases possibly due to the infected boy bottle-capper, but also subject to water infection; and the fact that there were only two out of a comparatively long list of customers makes it unlikely that the milk was the source; again nine cases occurred following the case of the milker mentioned above, but these also were exposed to water infection, and owing to the mixing of milk before sale, the milker could hardly have infected so small a number out of a large clientèle.

Finally, two cases developed early while using the milk of a small dealer where typhoid existed, and two more cases, developing long after the mass of the epidemic, were still using the same milk. The two later cases were brothers, and both the father and a sister had had typhoid and were then convalescent. It would be difficult to determine the milk as the cause of any of these.

Hence it may be concluded that milk had nothing to do with the primary outbreak and that no evidence has been obtained showing even secondary infection from milk, although the possibility of secondary infection in this manner must be allowed in a total of 21 cases, for most of which primary infection from the water was the more probable explanation.

POSSIBLE INFECTION THROUGH FLIES.

There were one or two secondary cases in which the investigation made gave small satisfaction as to the exact route of the infection from infector to infectee.

Flies as a carrier of typhoid fever, in a well-sewered community where the excreta are promptly disposed of into the sewer, should show few if any instances of fly infection from house to house although careless exposure of filled or unwashed bed pans in houses or on porches, etc., with fly access to them, may give occasional house infections or a limited number of house to house infections.

In Mankato unusual care as to disinfection and disposal of excreta was the rule and sewer connections were fairly constant in the city water district; hence it was not surprising that fly infection should prove the only available explanation in extremely few cases. The "clean up" of the city by the local health authorities undoubtedly minimized the breeding place of flies also.

WALKING CASES.

An "intensive" or house-to-house investigation, carried out by the medical inspectors and district nurses, covered 193 families taken as they came on certain streets in certain districts so selected as to average the conditions in the city as nearly as possible. This investigation was designed to discover the number of persons affected by initial diarrhea in families where typhoid did not occur and also to determine how thoroughly the orders of the health authorities regarding the boiling of water and milk were carried out. The results are probably far from accurate in individual cases but probably also represent the average condition fairly well. Incidentally, cases of possible mild or walking typhoid not reported by physicians were sought for.

RESULTS OF INTENSIVE INVESTIGATION.

Families = 193	Adults = 395	Children = 503
Families affected = 78	Adults = 78	Children = 104
Typhoid cases already reported = 19		
Possible walking cases discovered = 17		

For a variety of reasons, blood tests of the suspected walking cases were not made.

Thus of a total of 898 individuals included in this intensive investigation about one-fifth are recorded as having the initial diarrhea. This proportion is not so high as that derived from data taken from patients' families. But also the percentage of

typhoids among these families was smaller than the percentage of cases in the whole population (by about one-half). The explanation of both apparent deficits lies in the fact that a large number of the families here investigated were users of well-water and hence escaped the wholesale infection which occurred among those who drank city water exclusively.

The number of apparent walking typhoids nearly approximated the number of recorded cases in this part of the population, and hence this outbreak is apparently an example of the dictum established by eastern epidemiologists that the recorded (bed) cases of typhoid fever are in general equaled by the unrecorded (usually walking) cases.

SECONDARIES.

In an outbreak like that at Mankato, the cases derived from the original environmental source (in this instance water) are known as primaries—those derived more or less directly from these primaries, by more or less direct transfer of the patients' discharges to the prospective victim's mouth, by hands, food, milk, or by flies conveying discharges to food, milk, etc., are known as secondaries. Strictly speaking there is no logical distinction between primary and secondary, for both are infected only by receiving into their mouths the discharges of previous cases. Hence every typhoid case is secondary to some other previous case, and often, also, primary to some other subsequent case. But it is convenient to speak of the originals, *so far as traced*, as primaries, and of those traceable to the primaries, as secondaries.

Hence the primaries, in this instance, are those infected by drinking the city water, the secondaries, those derived from the primaries. In a few instances, secondaries gave rise to still other secondaries (tertiaries) but anyone who has clearly in mind the fact that typhoid fever (like the other communicable diseases) never arises *de novo*, but always traces itself back by an endless chain from patient to preceding patient, must appreciate that this term is not very valuable.

The distinction in Mankato between primaries and secondaries is clear enough at the extremes of time. Thus as indicated above, it is more than probable that all cases whose dates of earliest

symptoms were not later than the third week after June 25, i.e., not later than July 16, were unquestionably primaries. Owing to the extreme probability that the water was infected, at least to some extent, as late as July 9, cases developing symptoms within three weeks of this latter date, i.e., by July 30, are in all probability attributable to the water also, although some of these occurring late in July, especially where they were associated with cases sick early in July, might also be secondaries. Since there is some evidence that infection of the mains persisted until July 18, perhaps even the 25th, it was possible that primaries might occur as late as August 8 or even August 15. But that they would not occur in any great number, especially among the residents in Mankato, is guaranteed by the great fear of the water, inspired by constant and strenuous publicity. Where family contact or especially nursing the sick could be shown in cases developing after July 30, the cases are considered as probably secondary. On this basis there were in Mankato secondary males, 15; secondary females, 36, a total of 51.

In three females (Numbers 351, 246, 370) the evidence is quite inconclusive. For reasons given above, five others (females) might have been infected from water as well as by contact. In the cases of seven other females, the presumption is strong that they were secondaries, but the proof is not final. Thus a total of three males and 12 females have at least some doubt as to origin. The remaining 32 can hardly be questioned as certainly secondary.

For the purposes of the following classification it has been assumed that 15 males and 36 females were secondary.

AGE CLASSIFICATION OF MANKATO SECONDARIES (UNDER 21 YEARS OF AGE).

NUMBER AT EACH AGE UNDER 21

	Ages, 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	NUMBER OF CASES (MALE)																	
Males 15, adult males 6,	2	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0
	NUMBER OF CASES (FEMALE)																	
Females 36, adult females 17,	1	0	1	1	3	1	0	2	0	1	1	1	1	0	4	0	2	0

FORM OF EXPOSURE.

Nursing sick......	Males = 2 Females = 10	Family contact......	Males = 8 Females = 16
Flies............	Males = 1 Females = 1	Laundry............	Males = 0 Females = 2
Not given.........	Males = 4 Females = 7		

AGE CLASSIFICATION, OUTSIDE SECONDARIES.

	Years 3,	10,	11,	13,	15
Males, 7; adults, 3	1,	0,	0,	2,	1
Females, 11; adults, 7	0,	1,	1,	0,	2

FORM OF EXPOSURE.

Nursing sick	Males = 0; Females = 4	Family contact	Males = 6; Females = 3
Visiting sick	Males = 1; Females = 3		

NOTE.—One female (45), secondary, was evidently doubtful as to derivation from Mankato at all.

North Mankato presented two female secondaries only.

Thus the total secondaries, possible, probable, and certain were 71. They formed therefore nearly 14 per cent of the total cases. The 290 females yielded 49 secondaries, or 17 per cent. The 221 males yielded 22 secondaries, or 10 per cent.

The usual percentage of secondaries in most such outbreaks seems to be 25 per cent. Hence at least 51 secondaries were saved by the strenuous supervision and the publicity with which the public health work was done. This, however, is hardly a fair estimate, for this publicity acted chiefly in Mankato.

Of 405 cases in Mankato, 51 were possibly secondary, or 12.5 per cent, cutting the usual percentage in two. If only 32 be allowed as known secondaries the percentage in Mankato becomes 8.6 per cent.

Outside of Mankato, where these efforts did not extend, there were 84 cases, with 18 secondaries, or 21.5 per cent, which approximated the usual figure.

In North Mankato, the percentage is just under 10 per cent, but the number of cases was too small to be of much value and the influence of the publicity in Mankato undoubtedly extended to North Mankato.

Secondary infection within households, due to the infection of the nurse who is caring for the sick at home, or to "family contact," i.e., the fact that the nurse, trained, untrained, or perhaps a member of the family (usually the mother or elder daughter) carries the infection to her own mouth or to those of the family by transferring the discharges of the patient to food, milk, dishes, etc., and the direct transfer by a recovered member to the others, of

still-infected discharges, must be distinguished from secondary infection from outside the family. The latter is introduced by flies, by visiting the sick, or visitors from the sick, by visitors or associates suffering from unrecognized or walking cases, early mild cases, or convalescents; also by carriers or by infection of milk or other food coming into the family, by washing typhoid-infected clothes, etc.

Infection within the family is both more difficult and more easy to avoid than infection from outside the family—more difficult because of its concentration, the continuity of its presence, and inevitable carelessness due to exhaustion, worry, and hurry in the family, consequent on its presence; more easy because the routes of infection are so well known and the methods for preventing spread so simple; more difficult because these sources and routes must be unremittingly watched minute by minute; more easy because such watchfulness is possible as it cannot be with regard to the multiplicity of possibilities from without.

It is distressing to note even the number of trained nurses who infect themselves and therefore, doubtless, others. It is rather evident that increased training and increased caution is needed on the part of trained nurses as well as of untrained nurses.

The one teaching that typhoid fever is not contagious has been responsible for a very great number of cases and deaths, wholly and absolutely avoidable, and the avoidance was wholly in the control of those who became infected thus, had they but known or appreciated the facts. Perhaps the other most pernicious teaching regarding typhoid fever is that which relates its spread almost wholly to water supplies.

While it is true that, as this Mankato outbreak demonstrates, the great outbreaks which catch the public attention and are remembered are usually water outbreaks, yet the major portion of the total typhoid of Minnesota is not water typhoid, but due to contact, flies, and milk infection.

The State Board of Health has combated for years both of these pernicious teachings and at length is beginning to feel that they have impressed the public and professional mind to some extent.

THE FATALITY OF TYPHOID FEVER IN THIS OUTBREAK.

"Fatality," technically, is the figure expressing the proportion of deaths from any one disease occurring in a given series of cases of the same disease. Hence it is subject to two obvious fallacies—incorrectness in *death* returns, and incorrectness in the returns of *cases*. Entirely apart from the laxness with which cases seen by physicians are reported, the real total of cases is always incomplete because some cases are always concealed, some too mild to call for a physician, and some, being seen, are not recognized. It is customary among epidemiologists to consider that the average outbreak of most infectious diseases in an average community consists of two distinct parts, approximately equal in dimensions—the recognized cases and the "missed" cases, the latter divided into mild, unrecognized, and concealed cases.

Hence it follows that every epidemic disease possesses two gross fatalities: that based upon reported or "bed cases," and that, rarely ascertained accurately, but much more nearly expressing the truth, based upon the *total* cases. The latter fatality will be obviously about half of the former.

In ordinary practice, the death returns are much more nearly correct than the case reports. Hence has arisen a method of determining approximately the number of cases in a given epidemic or endemic by calculation from the deaths, the latter being multiplied by a figure supposed to represent "fatality." Thus the prevalence of typhoid fever in a given community during a given period is often estimated by multiplying the deaths from typhoid by 10, since it is sometimes true that about 10 per cent of typhoid cases die.

The Mankato outbreak was exceptional in that the case reports were practically as complete as the death reports, and both were practically as accurate as any such figures, outside of hospital practice, could be expected to be.

It was considered, therefore, worth while, despite the relative meagerness of the figures, to work out more than the mere gross fatalities.

These are appended. All are based (as will be readily seen) on reported or "bed cases." On the pretty well established hypothesis

that the "unseen" outbreak equals the "seen," each should be cut approximately in two to indicate the true facts.

It will be noted that applying the factor 10 to the deaths in Mankato would have yielded 350 as the calculated number of cases, which would have been 161 cases short of the truth; i.e., the calculated number would have required nearly 50 per cent increase before it would have approximated even the bed cases.

The calculations appended show that in this outbreak, adult males died in higher proportion than did any other group, while males under 19 died in smaller proportions than did any other group; moreover that the fatality of adults in general was more than double that of minors. It will be remembered that the relative age incidence of the disease indicated a comparative age-immunity to typhoid fever, especially up to the age of 10 years. This latter age-group constituting 27–30 per cent of the average population, yielded but 12 per cent of the total cases and but 6 per cent of the deaths.

Although possibly only a trick of small figures, it is interesting to note that the fatality rose as the outbreak progressed, being, for males, nearly four times as great in October as in July.

DEATHS FROM TYPHOID FEVER.

AGE AND SEX RELATIONS IN MANKATO OUTBREAK.

	Total Cases	Total Deaths	Percentage
Males	219	16	7.3
Females	292	19	6.5
	511	35	6.85

AGE 0–19 YEARS.

Males	111	4	3.6
Females	138	7	5.0
	249	11	4.4

AGE 20+ YEARS.

Males	108	12	11.1
Females	154	12	7.7
	262	24	9.1

Relation to Primary and Secondary Infection.

	Total Primary	Total Secondary	Deaths Primary	Deaths Secondary	Death-Rate Primary Percentage	Death-Rate Secondary Percentage
Males	197	22	14	2	7.1	9.0
Females	243	49	16	3	6.5	6.1
	440	71	30	5	6.8	7.0

RELATION TO STAGE OF OUTBREAK, I.E., INFECTION EARLY OR LATE.

Classification of the deaths by dates of earliest symptoms shows the following:

Total Cases by Dates of Earliest Symptoms.

	June	July	August	September	October
Male	0	186	19	6	4
Female	4	231	40	7	7
	4	417	59	13	11

Total Deaths by Dates of Earliest Symptoms.

	June	July Percentage	August	September Percentage	October Percentage
Male	0	12 6.4	0	1 16.6	1.25
Female	0	15 6.4	37.5	1 14.2	0.0

MALE DEATHS.

Age	Occupation	First Symptoms	Primary or Secondary	Died
?	?	?	P	New Ulm
2	Child	7/4	P	Mankato
15	Student	9/30	S	Mankato
18	Delivery	7/10	P	Mankato
21	Asst. mgr.	7/1	P	Mankato
22	Laborer	10/4	S	Mankato
24	Student	7/4	P	Mankato
26	Farmer	7/15	P	Vernon Center
28	Engineer	7/13	P	Mankato
30	Laborer	7/8	P	Mankato
32	Shp. clerk	7/14	P	Mankato
33	Banker	7/10	P	Mankato
35	Shipper	7/3	P	Mankato
37	Traveler	7/7	P	Mankato
40	Bookkeeper	7/12	P	Mankato
44	Salesman	?	P	Mankato

FEMALE DEATHS.

Age	Occupation	First Symptoms	Primary or Secondary	Died
7	Child	7/5	P	Mankato
14	Student	7/26	P	Mankato
16	Student	7/12	P	Mankato
17	Housewife	7/16	P	Mankato
18	Nurse	8/1	S	Mankato
19	Student	7/9	P	Mankato
19	Milliner	7/6	P	Mankato
21	Servant	7/9	P	Janesville
21	Student	7/11	P	Mankato
22	Student	7/18	P	Janesville
22	Teacher	7/7	P	Bricelyn
23	Housewife	7/7	P	Mankato
28	Housewife	8/12	P	Mankato
31	Nurse	8/15	S	Good Thunder
34	Housewife	7/13	P	Mankato
43	Dressmaker	7/14	P	Mankato
44	Housewife	7/10	P	Mankato
46	Housewife	7/10	P	Mankato
50	Housewife	9/28	S	Mankato

Age	Cases			Deaths			Percentage		
	Males	Females	Total	Males	Females	Total	Males	Females	Total
1–4	7	4	11	1	0	1	14.2	25.0	9.1
5–9	20	21	41	0	1	1	0.0	4.7	2.4
10–14	43	41	84	0	1	1	0.0	2.4	1.2
15–19	42	72	114	2	5	7	4.7	7.0	6.1
20–24	31	61	92	3	5	8	9.6	8.2	8.7
25–29	20	26	46	2	1	3	10.0	3.8	6.5
30–34	16	28	44	3	2	5	19.0	7.0	11.3
35–39	14	11	25	2	0	2	14.3	0.0	8.0
40–44	12	11	23	2	2	4	17.0	18.0	17.3
45–49	5	4	9	0	1	1	0.0	25.0	11.1
50–54	5	5	10	0	1	1	0.0	20.0	0.10
55–59	2	3	5	0	0	0	0.0	0.0	0.0
60–64	0	1	1	0	0	0	0.0	0.0	0.0
65–69	1	0	1	0	0	0	0.0	0.0	0.0

It would appear from these figures (which are far too small, however, for any reliable general deductions) that the fatality by age-groups was greatest at the extreme ages (1–4 and 30+) than at intermediate ages, the intermediate ages furnishing the mass of the cases. Hence typhoid fever, in this outbreak, like poliomyelitis, and unlike diphtheria and scarlet fever, showed its higher fatalities in the age-groups which yielded the fewer cases.

APPENDIX TO MANKATO REPORT.

TABLE I.

SHOWING ALL CASES BELONGING TO MANKATO OUTBREAK, BY DATE OF EARLIEST SYMPTOMS.

June	28th	29th
Mankato male, P.	0	0
Mankato female, P.	2	2

July	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Mankato male, P.	4	0	1	8	4	6	4	4	7	13	13	11	8	6	5	7	7	8	6	10	4	1	3	1	4	3	0	0	0	1	2	151
Mankato female, P.	5	0	1	6	2	9	6	8	9	16	14	16	11	11	5	15	6	8	5	6	5	5	1	5	4	1	0	2	0	0	0	182
Mankato female, S.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	2
North Mankato male, P.	1	0	0	0	1	1	0	0	1	0	1	1	0	1	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	11
North Mankato female, P.	0	0	0	1	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
North Mankato female, S.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Outside male, P.	1	0	0	2	1	1	1	0	0	3	0	5	2	0	3	0	2	0	0	0	0	0	1	0	1	1	0	0	0	0	0	24
Outside female, P.	2	0	0	0	2	5	3	2	3	4	3	1	3	0	2	0	1	4	0	1	1	0	0	1	0	0	0	0	0	0	0	38
Total Mankato, P.	9	0	2	14	6	15	10	12	16	29	27	27	19	17	10	22	13	16	11	16	9	6	4	6	8	4	0	2	0	1	2	333
Total North Mankato, P.	1	0	0	1	2	2	1	1	2	1	1	1	1	1	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	19
Total outside, P.	3	0	0	2	3	6	4	2	3	7	3	6	5	0	5	0	3	4	0	1	1	0	1	1	1	1	0	0	0	0	0	62
Total Mankato, S.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	2
Total North Mankato, S.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Grand total	13	0	2	17	11	23	15	15	21	37	31	34	25	18	16	22	16	23	11	17	10	6	5	7	9	5	1	2	0	2	3	417

August	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Mankato male, P.	3	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Mankato female, P.	2	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Mankato male, S.	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	6
Mankato female, S.	0	0	0	2	0	1	0	0	1	1	3	1	0	1	1	1	2	0	2	2	0	3	0	0	0	1	0	2	0	1	0	25
North Mankato male, P.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
North Mankato female, S.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Outside female, P.	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Outside male, S.	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	5
Outside female, S.	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	6
Mankato, P.	5	2	1	0	2	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
Mankato, S.	0	0	0	2	0	2	1	1	1	1	3	1	0	1	1	1	2	0	2	2	0	3	0	2	0	2	0	2	0	1	0	31
North Mankato, P.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
North Mankato, S.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Outside, P.	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Outside, S.	0	1	0	1	0	1	0	0	1	1	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	11
Total, P.	7	2	1	0	2	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
Total, S.	0	1	0	3	0	3	1	1	2	2	3	2	1	1	2	1	2	1	2	3	0	3	1	2	0	2	0	2	1	1	0	43
Grand total	7	3	1	3	2	3	1	2	2	3	3	3	1	1	2	2	2	1	2	3	0	3	1	2	0	2	0	2	1	1	0	59

TABLE 1—*Continued.*

September	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
Mankato male, S.	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1		5
Mankato female, S.	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0		3
Outside male, S.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		1
Outside female, S.	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0		4
Mankato, S.	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1		8
Outside, S.	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1		5
Grand total	1	1	1	0	0	2	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	2		13

October	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Mankato male, S.	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3
Mankato female, S.	1	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Outside male, S.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Outside female, S.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Mankato, S.	1	0	0	2	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	9
Outside, S.	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Total	1	0	0	3	2	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	11

	Total Cases Listed	Primary	Secondary	Male	Female
June	4	4	0	0	4
July	417	414	3	186	231
August	59	16	43	19	40
September	13	0	13	6	7
October	11	0	11	4	7
	504	434	70	215	289

CASES OMITTED WITH REASONS.

Male, Primary 133 Mankato; Date not given. (July?)
Male, Primary 262 " " " " (July?)
Male, Primary 134 " " " " (July?)
Male, Secondary 322 " " " "
Male, Primary 51 Outside. No evidence. (Probably sick in Mankato)
Male, Primary 69 " Date not given. (July)
Female, Primary 398 Mankato. " " " (July early.)

TABLE 2.

AGE AND SEX OF PATIENTS—MANKATO OUTBREAK.

Age	Male: North Mankato Total	Male: Outside Total	Male: Mankato Total	Male: Males Total	Comparison with Female Table Below: Females Total	Females Excess	Excess Made Up Thus: North Mankato	Outside	Mankato
Under 1	0	0	6	0	0	0			
1– 4	0	1	6	7	4	− 3			
5– 9	1	0	19	20	21	+ 1			
10–14	0	4	39	43	41	− 2			
15–19	1	5	36	42	72	+30	+1	+13	+16
20–24	3	8	20	31	61	+30	+2	+ 7	+21
25–29	2	4	14	20	26	+ 6	−2	+ 4	+ 4
30–34	2	4	10	16	28	+12	−2	− 2	+16
35–39	3	3	8	14	11	− 3			
40–44	0	1	11	12	11	− 1			
45–49	0	1	4	5	4	− 1			
50–54	0	0	5	5	5	0			
55–59	0	0	2	2	3	+ 1			
60–64	0	0	0	0	1	+ 1			
65–69	0	0	1	1	0	− 1			
	12	31	175	218					
		* 2	* 1	3					
		33	176	221					

FEMALE

Age	North Mankato Total	Outside Total	Mankato Total	Total
Under 1	0	0	0	0
1– 4	0	0	4	4
5– 9	0	0	21	21
10–14	2	4	35	41
15–19	2	18	52	72
20–24	5	15	41	61
25–29	0	8	18	26
30–34	0	2	26	28
35–39	0	1	10	11
40–44	0	1	10	11
45–49	0	1	3	4
50–54	1	0	4	5
55–59	0	1	2	3
60–64	0	0	1	1
65–69	0	0	0	0
	10	51	227	288
			2	2
			229	290

* Ages not given.

Male (51 outside) "boy."

Male (69 outside) "adult."

Male (133 Mankato) "boy."

Female (598 Mankato) "young."

Female (248 Mankato) "adult."

TABLE 3.

OCCUPATIONS OF MANKATO CASES.

Normal-school students	9
Other students	84
Normal-school teachers	4
Music teachers	2
Other teachers	7
"At home"—i. e., young children, young men and women not working, a few children of school age not attending school, a few old people unfit for work, etc.	82
Housewives	72
Nurses—hospital	6
Nurses—private	3
Domestics	5
Cook—hotel	1
Cook—café	1
Kitchen help, café	1
Waitress, lunchroom	1
Grocers	1
Bookkeeper	1
Clerks	13
Shipper	4
Bakers	2
Candy-makers	6
Laundry	6
Druggists	2
Milker	1
Capper	1
Butchers	2
Steam-fitter	1
Bookkeepers	3
Stenographers	4
Salesmen	3
Collector	1
Laborers	11
Milliners	6
Tailor	1
Knitters	5
Paper-hanger	1
Janitor	1
Janitress	1
Teamsters	4
Livery	1
Photographers	3
Gas-fitter	1
Bankers	3
Cigar-makers	2
Coopers	3
Carpenters	3
Bellboys	2
Barbers	2
Street Railroad	1
Furniture	1
Convent House	1
St. Joseph's Hospital	1
Engineers	2
Real estate	2
Actress	1
Railroad men	2
Brick-layer	1
Pressroom	1
Plumber	1
Preacher	1
Shoemaker	1
Physician	1
Merchant	1
Bookbinder	1
Lumberyard	1
Malster	1
Miller	1
Stone-cutters	2
Life insurance	1
Not given	6

TABLE 4.

OCCUPATIONS OF OUTSIDE CASES WITH REASONS FOR BEING IN MANKATO.

PRIMARIES.

Attending Circus.

Students	7
Housewives	3
Farmers	4
Teachers	2
Laborer	1
Butter-maker	1
	18

Visitors.

Students	3
Housewives	7
Farmers	4
Teachers	2
Ministers	3
Banker	1
Lumberman	1
Not given	2
	23

Temporary Business.

Domestics	6
Clerk	1
Carpenter	1
	8

Studying in Mankato.

Students, Normal	8
Teachers	5
Other students	4
	17

Total	66

SECONDARIES TO OUTSIDE CASES.

To Housewives.

Farmer	1
Students	3

To Minister.

Housewife	1
Students	3

To Farmers.

Housewife	1
Housewife	1
Sister	1
Brother	1
Husband	1

SECONDARIES TO CASES IN MANKATO.

To Patient in Mankato.

Nurse	1
Secondary to this nurse	1

To Daughter in Mankato.

Housewife	1

To Brother in Mankato.

Housewife	1

To Unknown Source.

Student	1
Total	18

TABLE 5.

OCCUPATIONS, NORTH MANKATO CASES.

Students	3	All drank water in Mankato, at circus, carnival, or party.
Laborers	3	All worked in Mankato.
Clerks	3	" " " "
Carpenters	4	" " " "
Roofer	1	" " " "
Shipper and helper	2	" " " "
Knitter	2	" " " "
Cabinet-maker	1	" " " "
Candy-packer	1	" " " "

SECONDARIES.

Housewife, nursing daughter	1
Housewife, no history	1

TABLE 6.

OUTSIDE PLACES INVOLVED IN MANKATO OUTBREAK.

(Unless otherwise stated, these places were in Minnesota.)

Places.	*Cases.*		*Places.*	*Cases.*	
Amboy	1	(farm near 1)	New Ulm	2	
Austin	1		Ottawa	2	
Bricelyn	1		Owatonna	2	
Cambria	1		Rapidan		(farm near 4)
Columbus, N. D.	1		Red Wood Falls	1	
Decoa	1		Ryder, N. D.	1	
Eagle Lake	1		St. Clair		(farm near 6)
Easton	1		St. Peter	3	
Fairfax	1		Seattle, Wash	1	
Good Thunder		(farms near 10)	South Bend		(farm near 1)
Holloway	1		Springfield		(farm near 1)
Janesville		(farms near 6)	Staples	1	
Judson		(farms near 2)	Towner, N. D.	1	
Kasota	1	(farm near 3)	Trueman	1	
Lake Crystal	1		Vernon Center		(farm near 1)
Le Sueur		(farm near 1)	Waterville	3	
Mankato		(farm near 4)	Wells	1	
Mapleton		(farm near 2)	White, S. D.	5	
Morgan	2		Willmar	1	
Nicollet		(farm near 1)	Winona	2	

Cases sick in Mankato, but belonging outside	Cedilla, Mich.	1	=7
	Bigelow	1	
	Minneapolis	1	
	Jackson	1	
	New Richland	1	
	New Prague	1	
	Manson, Iowa	1	

Places 40 (outside Mankato and North Mankato)
Hence 49 places in all affected.
84 cases outside Mankato and North Mankato.
7 cases sick in Mankato from outside pcints.
—
91 cases.

TABLE 7.

OUTSIDE PLACES SHOWING SECONDARIES (18).

One near Rapidan was possibly an extraneous case.
(NOTE.—One case was a secondary from Mankato and infected further case at home.)

Janesville	1	Infected by sisters, primaries from Mankato.
White, S. D.	4	" " brother-in-law, a primary from Mankato.
Good Thunder	4	" " sister, a primary from Mankato.
Good Thunder	2	" " sister, a secondary from Mankato.
Rapidan	1	" " brother, a primary from Mankato.
St. Clair	2	" " sister, a primary from Mankato.
St. Clair	1	" " daughter, a primary from Mankato.
Near Mankato	1	" " wife, a primary from Mankato.
Willmar	1	" " brother, a primary in Mankato.

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